Conservation Genetics of the Capercaillie (*Tetrao urogallus* L.) in the Swiss Alps

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Gwenaël Jacob

aus

Frankreich

Promotionskomitee

Prof. Dr. Bernhard Schmid (Leitung der Dissertation)

Prof. Dr. Lukas Keller

Prof. Dr. Jacob Höglund

Dr. Felix Gugerli

Dr. Kurt Bollmann

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Chapter 1

General introduction

Application of genetic studies in conservation biology

Conservation genetics encompasses to manage small populations for maximising the retention of genetic diversity and minimizing inbreeding, to resolve taxonomic uncertainties and delineate management units, and to use molecular techniques for understanding the biology of the study species (Frankham et al. 2002).

Habitat change and fragmentation result in a decreasing number and size of suitable habitat patches and in increasing geographic distances between the remaining patches, which is considered a major threat for species persistence in altered landscapes (Andrén 1994; Fahrig 1997). Small and isolated populations face a high risk to go extinct by stochastic events, *e.g.* when habitat patches are destroyed (Lande 1993), or by lacking the genetic diversity required to adapt to a changing environment (Lande 1994; Lande & Shannon 1996). Moreover, mating with relatives, which is likely to occur in small and isolated populations, may decrease the individuals' reproductive success and survival (Lande 1994; Saccheri et al. 1998). Management actions directed towards restoring gene flow between populations, *e.g.* individuals' translocation, may enhance population growth or recovery (Hedrick 1995; Madsen et al. 1996; Westemeier et al. 1998). However, translocated individuals bear the risk of introducing new diseases, which may further threaten the population under management. Several experiments with plants showed that crossing individuals from populations separated each other by large distances may lower the fitness of the offspring, so called outbreeding depression (*i.e.*, Fischer & Matthies 1997), but this phenomenon has rarely been observed in wild animal populations (Lynch 1991; Frankham 1995).

Both landscape permeability and ecological factors may influence the genetic connectivity between populations, and it is relevant to conservation practices to delineate management units for endangered species. Habitat corridors may favour individual movements between habitat patches separated from each other by large distances and enhance the genetic relatedness between populations (Haas 1995; Coulon et al. 2004). In contrast, landscape barriers may constrain gene flow and induce substantial genetic differentiation between neighbouring populations so that they are considered as different management units (Paetkau et al. 1999). Similarly, mechanisms of reproductive isolation may prevent admixture between populations that persisted in different refugia during glacial periods or followed different colonisations routes (Taberlet et al. 1998; Irwin et al. 2001; Lugon-Moulin & Hausser 2002). Selective pressure for secondary sexual traits may also induce minor differences in behaviour or morphology, *e.g.* song or feather patterns, between neighbouring populations which may act as ecological barriers to gene flow (Omland et al. 2000; MacDougall-Shackleton & MacDougall-Shackleton 2001). By allowing the detection of barriers to gene flow between populations or groups of individuals, genetic studies (in combination with other ecological studies) provide a valuable tool to delineate management units (Moritz & Faith 1998).

Non-invasive genetic studies may provide a valuable alternative to telemetry or mark–recapture to investigate the biology of threatened species and to elaborate appropriate management plans, especially if the species deserves urgent management actions. Examples of such applications include studying group structure and mating systems (Amos et al. 1993; Morin et al. 1993), tracking individuals for enumeration (Taberlet et al. 1997) and estimating population census sizes (Kohn et al. 1999). Recently, Rudnick et al. (2005) monitored the population turnover in Eastern Imperial Eagle (*Aquila heliaca* Savigny) from shed feathers, thus demonstrating the potential of genetic studies in conservation biology.

The development of molecular techniques in conservation biology

The past 20 years have seen three major innovations in molecular methods. First, the polymerase chain reaction (PCR) was developed, by which millions of copies of a single genomic nucleotide sequence can be obtained for analyses (Saiki et al. 1988). The PCR allows using picograms of DNA as template for amplification, which has considerably eased the use of molecular techniques in population biology. Second, the discovery and isolation of short tandem sequence repeats (Tautz 1989) provided researchers with highly polymorphic

genetic markers. Neutral microsatellite loci, *i.e.* those that are situated in regions of the genome not under selection, provide ideal markers to study the genetic relationship between populations. Finally, improvements in the procedures of DNA preservation, extraction and amplification made it possible to use samples collected non-invasively as sources of DNA for molecular analyses (Woodruff 1993; Palsbøll et al. 1997; Taberlet et al. 1997).

Thus, the rapid development of the molecular techniques allowed minimizing the disturbance induced by the researchers, which is of particular relevance to the study and conservation of endangered species. Moreover, non-invasive genetic techniques do not require direct contact to the study animals, which greatly eased the study of rare and elusive species.

The study species

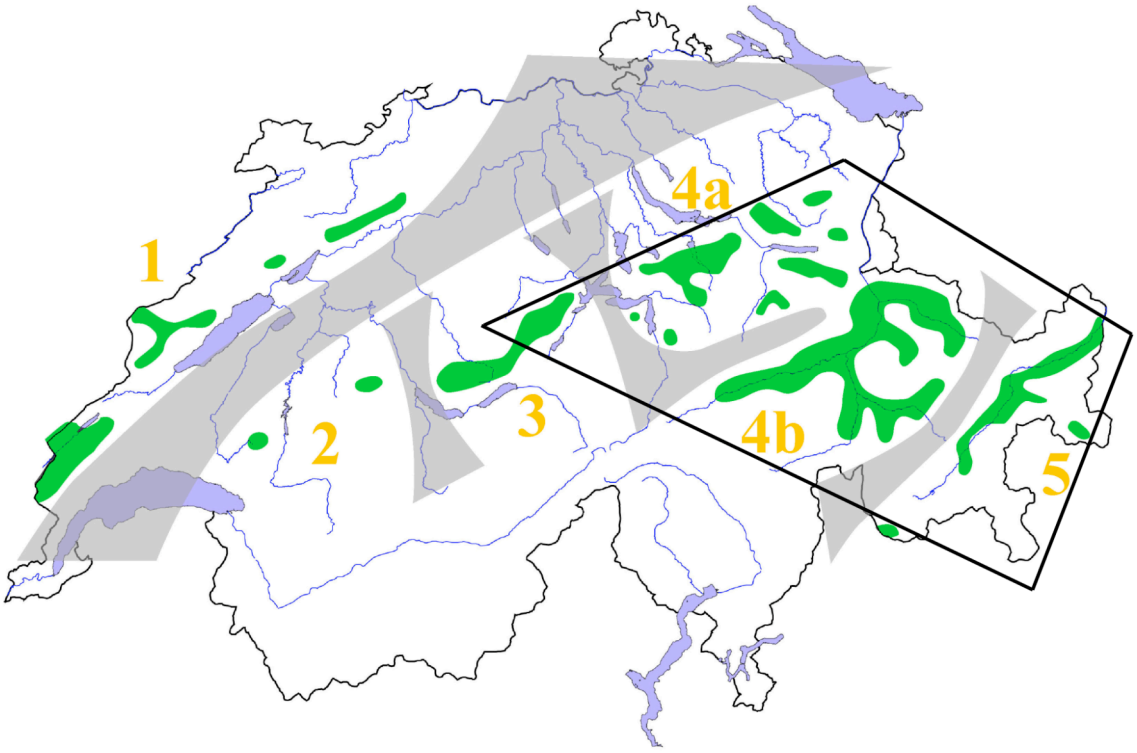
The capercaillie belongs to the avian subfamily Tetraoninae (Galliformes, Phasianidae), which includes 18 recognised species in seven genera (Storch 2000). Of these, the genus *Tetrao* is represented by four species: the Western capercaillie (*T. urogallus* L.) and its sister species the Black-billed capercaillie (*T. parvirostris* Bonaparte), and the Eurasian black grouse (*T. tetrix* L.) and its sister species the Caucasian black grouse (*T. mlokosiewiczi* Taczanowski). The Western capercaillie (hereafter capercaillie) is a large forest grouse species with its main distribution range in the taiga-like forests of Scandinavia and Russia. Outside of this range, the capercaillie also occurs in mixed-deciduous and coniferous forests of mountainous areas in western and central Europe (Storch 2001). Twelve subspecies have been described based on morphological, behavioural, ecological and biogeographical features (del Hoyo et al. 1994). Of these, three subspecies inhabit geographically isolated mountain ranges in western and central Europe: *T. u. cantabricus* occurs in the Cantabrian Mountains, *T. u. aquitanicus* in the Pyrenees and *T. u. rudolfi* in the Carpathians Mountains. The subspecies considered in this study, *T. u. major*, is distributed in the Alps, in Poland, in southwest Scandinavia and in Scotland, where birds of Swedish origin were released to re-establish a sustainable population.

The capercaillie shows lekking behaviour, that is, mating occurs on arenas situated at exposed sites in open forest stands or mires, *i.e.* leks, on which males and females aggregate

in late winter. Males generally converge earlier than do females on the leks, where they occupy and defend small territories during the breeding season, *i.e*. from late winter to mid spring. This territorial behaviour is age-related, and males 4 years old and older defend the smallest territories positioned around the lek, whereas 1-2 years old males generally occupy marginal territories and may visit several leks during the breeding season (Wegge & Larsen 1987; Storch 1997). Females usually stay further away from the leks than do males and visit one or several leks during brief periods (Wegge & Rolstad 1986; Storch 1997). Radio-tracking studies conducted in central Europe and Scandinavia showed that males are mostly philopatric and females tend to disperse up to 5-10 km (review in Storch 2000). These observations have been confirmed by genetic studies, which showed that males found at the same leks tended to be more related than males originating from different leks (Regnaut et al. 2006). Male philopatry suggests, in turn, that female-biased dispersal may occur in the capercaillie, as shown in the two related species also showing lekking behaviour, the Greater prairie-chicken, *Tympanuchus cupido* L. (Johnson et al. 2003) and the red grouse, *Lagopus lagopus scoticus* L. (Piertney et al. 2000). The annual home range of females is *c*. 550 ha in the Bavarian Alps (Storch 1995), although females visiting several leks may have larger annual home ranges (Storch 1997).

Since the beginning of the 20th century, the population of *T. urogallus* in central Europe has declined, mostly because land use and forestry practices have changed (Helle et al. 1994; Storch 2000; 2001). Moss et al. (2001) showed that the breeding success in the Scottish capercaillie decreased as a result of delayed spring warming during the period 1975–99. This result suggests that climate change may also impact the dynamics of the capercaillie in other areas of the distribution range of the species in Europe. The capercaillie population in Switzerland experienced a pronounced and continuous decline in distribution range and numbers since the 1950–60s (Marti 1986; Mollet et al. 2003), reading a current size of *c*. 450–500 males (Mollet et al. 2003). The species is nowadays restricted to mixed-deciduous and coniferous forests in mountainous areas. Mollet et al. (2003) suggested that the extant *T. urogallus* population in Switzerland is divided into five geographically isolated regions: the Jura Mountains (1), the western Prealps (2), the central Prealps (3), the eastern Prealps–central Alps (4a and 4b), and the Engadin and the southern valleys of the canton of Grisons (5). These regions are separated from each other by landscape barriers such as the central plateau or high mountain ranges (Fig. 1).

Figure 1: *Distribution of* Tetrao urogallus *in Switzerland (dark shadings), putative barriers to gene flow (light shadings) and spatial partition into five capercaillie regions: the Jura Mountains (1), the western Prealps (2), the central Prealps (3), the eastern Prealps–central Alps (4a and 4b) and the Engadin valley and the southern valleys in the canton of Grisons (5). Map from Mollet et al. (2003). Our study area is indicated by the dark straight line.*



Following the general decline of the capercaillie observed in Europe, conservation measures for the species have been proposed in the Grouse status survey and action plan 2000–2004 (Storch 2000). In this issue, Storch and co-authors stressed the gap in knowledge of the minimum requirements in population size, and in habitat patch size and connectivity. These factors are relevant to conservation in the regions where the species is endangered. However, several factors may affect the persistence of the capercaillie in a fragmented landscape, including the size and distribution of the habitat patches, the dynamics of the local populations and the level of genetic diversity maintained within the local populations. This

highlights the need for multi-disciplinary approaches to study the factors influencing the persistence of the capercaillie at the landscape level. A research project was initiated in 2001 at the Swiss Federal Research Institute WSL that aimed at investigating the capercaillie dynamics in the Swiss Alps by linking landscape ecology, population biology and population genetics studies (Bollmann 2002). Within the project, genetic studies were planned to estimate the census sizes in local capercaillie populations, to infer the genetic relationship between and the genetic diversity within local capercaillie populations, to identify landscape barriers that constrained exchange between local capercaillie populations and to compare historic and extant levels of genetic diversity. Conclusions of the different research fields will be integrated into a model to predict the dynamics of the capercaillie in the Swiss Alps and to elaborate an action plan for the management and conservation of the capercaillie in Switzerland (Mollet in prep.).

The study area

Three capercaillie regions as described by Mollet et al. (2003) were included in our study area (Fig. 1). From our prior knowledge on the distribution of suitable habitat for the capercaillie and on direct and indirect observations, we suspected that the three capercaillie regions studied would host one or more core areas of the species' distribution in the Swiss Alps. These three regions differed greatly with respect to topography, habitat fragmentation at the landscape scale and vegetation cover (Bollmann et al. 2005; Graf 2005), and they represented a gradient from Prealpine to Alpine ecological conditions and habitat requirements for the capercaillie. Data were collected within the same study area for the three different modules of the project, *i.e.* landscape ecology, population biology and population genetics, to ease linking results from the particular modules (Bollmann 2002).

Thesis objectives

In the present thesis, I focused on three topics that are relevant to elaborate a management plans for the capercaillie in the Swiss Alps: (i) estimating the number of individuals in local populations, (ii) estimating the genetic variability within and inferring the genetic relationship among local populations and (iii) comparing the level of genetic variability between individuals collected before the pronounced and continuous decline of the species in the Swiss Alps since the 1960–70s and individuals collected during the period

2001–2004. I also assessed the feasibility of the genetic monitoring of the capercaillie in the Swiss Alps (iv), as an alternative to field census to evaluate the success of management actions.

Prior to the population genetic analyses, my first objective was to develop routine procedures to extract and amplify DNA from non-invasive samples. Protocols for DNA extraction from faecal and feather samples were set up aiming at controlling cross-sample contaminations. They are detailed in the Annex I. By combining the twelve microsatellite markers used in the present study into four PCR-multiplexes, I aimed at reducing the cost per sample analysed and, thus, at maximizing the set of samples analysed. I also set up a genotyping procedure that optimally allocate resources to samples that had the greatest prospect of being amplified at the molecular markers used in the present study.

Accurately estimating the number of individuals is a prerequisite to elaborate management plans for a species. In chapter 2, I used data inferred from non-invasive sampling techniques and molecular genotyping to estimate the number of individuals present in eleven local capercaillie populations in the Swiss Alps. These results were compared with the census sizes estimated during the field survey of the species in the Swiss Alps to evaluate the benefits and limitations of the field and genetic approaches and to elaborate recommendations for future monitoring projects.

Management units may be delineated by inferring the genetic structure and the population dynamics of the study species. In chapter 3, I investigated the genetic relationship among and the genetic diversity within local capercaillie populations in the Swiss Alps. These results were used to identify regions of importance for the sustainability of the capercaillie population in the Swiss Alps and to assign priority levels for management actions. In chapter 4, I investigated the consequences of the recent reduction in the species' distribution range and numbers on the genetic variability maintained in the present populations. For this, we compared the level of genetic variability in museum specimens, dated before the capercaillie's pronounced and continuous decline since the 1970s, and in the extant individuals. These results were used to determine the consequences of the recent species' decline on the connectivity among neighbouring areas of the capercaillie's distribution range in the Swiss Alps.

Managers need monitoring tools to evaluate the success of the management actions and to monitor population parameters, such as the individual reproductive success and

dispersal behaviour, and the population turnover. In chapter 5, I assessed the feasibility of an individual-based monitoring program for the capercaillie using non-invasive sampling techniques and molecular markers. By conducting this pilot study, I aimed at assessing the optimal sampling strategies and genetic analyses for the genetic monitoring of the capercaillie in Switzerland.

In chapter 6, I synthesize the results of the genetic analyses of the capercaillie in the Swiss Alps, and I conclude with recommendations for the conservation program of the species in Switzerland and in central Europe.

References

Amos B, Schlötterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, **260**, 670-672.

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos*, **71**, 355-366.

Bollmann K (2002) Dynamics of capercaillie in the Swiss Alps: Metapopulation modelling at the landscape scale - a project description. *Grouse News*, **23**, 6-7.

Bollmann K, Weibel P, Graf RF (2005) An analysis of central Alpine capercaillie spring habitat at the forest stand scale. *Forest Ecology and Management*, **215**, 307-318.

Coulon A, Cosson JF, Angibault JM, Cagnelutti B, Galan M, Morellet N, Petit E, Aulagnier S, Hewison AJM (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**, 2841-2850.

del Hoyo J, Elliott A, Sargatal J (1994) *Handbook of the Birds of the World*. Lynx Ediciones, Barcelona, Spain.

Fahrig L (1997) Relative effects of habitat loss and fragmentation on population extinction. *Journal of Wildlife Management*, **61**, 603-610.

Fischer M, Matthies D (1997) Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). *Am. J. Bot.*, **84**, 1685-1692.

Frankham R (1995) Conservation genetics. *Annu. Rev. Genet.*, **29**, 305-327.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to conservation genetics.* Cambridge University Press, Cambridge.

Graf RF (2005) Analysis of capercaillie habitat at the landscape scale using aerial photographs and GIS. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Zurich.

Haas CA (1995) Dispersal and use of corridors by birds in wooded patches on an agricultural landscape. *Conservation Biology*, **9**, 845-854.

Hedrick PW (1995) Gene flow and genetic restoration - the Florida Panther as a case-study. *Conservation Biology*, **9**, 996-1007.

Helle P, Helle T, Lindén H (1994) Capercaillie (*Tetrao urogallus*) lekking sites in fragmented Finnish forest landscape. *Scandinavian Journal of Forest Research*, **9**, 386-396.

Irwin DE, Bensch S, Price TD (2001) Speciation in a ring. *Nature*, **409**, 333-337.

Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology*, **12**, 3335-3347.

Kohn MH, York EC, Kamradt DA, Haugt G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. *Proc. R. Soc. Lond. B*, **266**, 657-663.

Lande R (1993) Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.*, **142**, 911-927.

Lande R (1994) Risk of population extinction from fixation of new deleterious mutations. *Evolution*, **48**, 1460-1469.

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, **50**, 434-437.

Lugon-Moulin N, Hausser J (2002) Phylogeographical structure, postglacial recolonization and barriers to gene flow in the distinctive Valais chromosome race of the common shrew (*Sorex araneus*). *Molecular Ecology*, **11**, 785-794.

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, **45**, 622-629.

MacDougall-Shackleton EA, MacDougall-Shackleton SA (2001) Cultural and genetic evolution in mountain white-crowned sparrows: Song dialects are associated with population structure. *Evolution*, **55**, 2568-2575.

Madsen T, Stille B, Shine R (1996) Inbreeding depression in an isolated population of adder *Vipera berus*. *Biological Conservation*, **75**, 113-118.

Marti C (1986) Verbreitung und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz. *Orn. Beob.*, **83**, 67-70.

Mollet P (in prep.) Aktionsplan Auerhuhn 2005 – 2020. Schweizerische Vogelwarte, Sempach.

Mollet P, Badilatti B, Bollmann K, Graf RF, Hess R, Jenny H, Mulhauser B, Perrenoud A, Rudmann F, Sachot S, Studer J (2003) Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. *Orn. Beob.*, **100**, 67-86.

Morin PA, Wallis J, Moore JJ, Chakraborty R, Woodruff DS (1993) Noninvasive sampling and DNA amplification for paternity exclusion, community structure, and phylogeography in wild Chimpanzees. *Primates*, **34**, 347-356.

Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*, **7**, 419-429.

Moss R, Oswald J, Baines D (2001) Climate change and breeding success: decline of the capercaillie in Scotland. *Journal of Animal Ecology*, **70**, 47-61.

Omland KE, Tarr CL, Boarman WI, Marzluff JM, Fleischer RC (2000) Cryptic genetic variation and paraphyly in ravens. *Proc. R. Soc. Lond. B*, **267**, 2475-2482.

Paetkau D, Amstrup SC, Born EW, Calvert W, Derocher AE, Garner GW, Messier F, Stirling I, Taylor MK, Wiig O, Strobeck C (1999) Genetic structure of the world's polar bear populations. *Molecular Ecology*, **8**, 1571-1584.

Palsbøll PJ, Allen J, Berube M, Clapham PJ, Feddersen TP, Hammond PS, Hudson RR, Jorgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjonsson J, Sears R, Smith T, Sponer R, Stevick P, Oien N (1997) Genetic tagging of humpback whales. *Nature*, **388**, 767-769.

Piertney SB, MacColl ADC, Bacon PJ, Racey PA, Lambin X, Dallas JF (2000) Matrilineal genetic structure and female-mediated gene flow in red grouse (*Lagopus lagopus scoticus*): an analysis using mitochondrial DNA. *Evolution*, **54**, 279-289.

Regnaut S, Christe P, Chapuisat M, Fumagalli L (2006) Genotyping faeces reveals facultative kin association on capercaillie's leks. *Conservation Genetics*, **in press**.

Rudnick JA, Katzner TE, Bragin EA, Rhodes OE, Dewoody JA (2005) Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Molecular Ecology*, **14**, 2959-2967.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491-494.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487-491.

Storch I (1995) Annual home ranges and spacing patterns of Capercaillie in Central Europe. *Journal of Wildlife Management*, **59**, 392-400.

Storch I (1997) Male territoriality, female range use, and spatial organisation of capercaillie *Tetrao urogallus* leks. *Wildlife Biol.*, **3**, 149-161.

Storch I (2000) Grouse Status Survey and Conservation Action Plan 2000-2004. (ed. Group WBSGS), p. 112 pp. IUCN, Gland, Switzerland and Cambridge, UK and the World Pheasant Association, Reading, UK.

Storch I (2001) *Tetrao urogallus* Capercaillie. *BWP Update*, **3**, 1-24.

Taberlet P, Camarra J-J, Griffin S, Uhres E, Hanotte O, Waits LP, Dubois-Paganon C, Burke T, Bouvet J (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Molecular Ecology*, **6**, 869-876.

Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson J-F (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453-464.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, **17**, 6463-6471.

Wegge P, Larsen BB (1987) Spacing of adult and subadult male Common capercaillie during the breeding-season. *Auk*, **104**, 481-490.

Wegge P, Rolstad J (1986) Size and spacing of capercaillie leks in relation to social behavior and habitat. *Behav. Ecol. Sociobiol.*, **19**, 401-408.

Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695-1698.

Woodruff DS (1993) Noninvasive genotyping of primates. *Primates*, **34**, 333-346.

Chapter 2

Estimating the population census sizes of the capercaillie (*Tetrao urogallus* L., Aves) in the Swiss Alps from single-occasion genetic capture-recapture data

Gwenaël Jacob*[1,2], Rolf Debrunner[1], Felix Gugerli[1], Bernhard Schmid[2] and Kurt Bollmann[1]

[1] Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

[2] Institute of Environmental Sciences, University of Zurich, Switzerland

Abstract

The capercaillie (*Tetrao urogallus* L.) has experienced a dramatic decline in Central Europe and been extirpated from large areas of its former natural range. In Switzerland, an action plan was initiated to evaluate the species' habitat requirements and demographic status and to decide for the appropriate strategy to maintain the species. During our study, we analysed 530 feather and faecal samples at twelve nuclear microsatellite loci and a sex-specific nuclear gene fragment. The multilocus genotypes grouped into 151 unique allelic combinations at those loci that were amplified. The distribution range of the samples was divided into 26 local populations. The number of males in those local populations was estimated from the distribution and quality of indirect and direct evidences of the species presence. We analysed samples collected during the field surveys to estimate the census sizes in eleven of those local populations, in which the sample size was largest, from

capture–recapture genetic data. Because samples were collected on single occasions, we estimated the census sizes using rarefaction models. We compared the census size estimates and the accuracy of four published algorithms to those from the field survey. We found that (i) two of those models were suitable for the estimation of the census sizes in small populations, (ii) the field survey tended to underestimate the census sizes in the capercaillie populations investigated, and (iii) the capture–recapture estimates were less influenced by the prior knowledge of the capercaillies' distribution in the local populations.

Keywords - capercaillie; census size estimate; nuclear microsatellites; non-invasive sampling, rarefaction method

Introduction

Habitat destruction and fragmentation have been considered as the two greatest threats for the sustainability of ecosystems worldwide (Andrén 1994; Bascompte & Solé 1996; Travis 2003). Consequently, an increasing number of species live in a fragmented environment and face a high risk of local extinction from demographic or environmental stochastic events (Lande 1993). Therefore, estimating the size of populations has become a major issue for monitoring the demography of endangered species in response to management action (Banks et al. 2003; Fisher et al. 2000; Franzreb 1997; Fujiwara & Caswell 2001; Maschinski et al. 1997).

Mark-recapture studies have been used to estimate the census sizes of wild populations (review in Pine et al. 2003; review in Schwarz & Seber 1999). However, these studies might not be appropriate for the study of endangered species because of their invasive nature, which might harm individuals (Kreeger et al. 1990; Vanballenberghe 1984). The advancement of sampling procedures and genetic techniques made it possible to use material dropped by individuals in the field as sources of DNA and to distinguish among individuals based on their genotypes (Gagneux et al. 1997; Gerloff et al. 1995; Morin et al. 1993; Taberlet & Luikart 1999). The number of unique genotypes identified, *i.e.* the minimum number of individuals alive (*MNA*), might be used as a first approximation of the census size. However, *MNA* is biased in most field conditions and might not be suitable to monitor wild populations (Mills et al. 2000).

In the last ten years, a number of models have been applied to estimate the census sizes of wild populations, from genetic-tagging data collected in a single capture occasion (Eggert et al. 2003; Frantz et al. 2004; Kohn et al. 1999; Miller et al. 2005; Wilson et al. 2003). Kohn *et al.* (1999), Eggert *et al.* (2003) extrapolated to the census sizes of populations by estimating the asymptotic value of accumulation curves fitted to the plots of the number of unique genotypes against the number of samples analysed. This approach was initially developed to estimate the species richness of an area (Colwell & Coddington 1994). Wilson *et al.* (2003) applied a model, reported in Valière (2002), that estimated the census sizes of populations from the distribution of the frequencies of capture per individual. Recently, Miller et al. (2005) released a program to estimate the census sizes of small populations based on an urn model with replacement. These authors implemented one model to account each for even or uneven frequencies of capture among individuals. No consensus came out on which model performs best in which situation, and additional simulation studies are still required to compare the performance of those five models in a wide range of sampling intensity and populations sizes.

Microsatellite markers have been developed for an increasing number of species and cross-species amplification allows to use loci identified in related taxa. Still, the number of polymorphic markers available for endangered species is often limited and there is the risk that two individuals share the same genotype (Mills et al. 2000). This "shadow effect" results in considering an individual not previously captured as recapture event.

Using non-invasive samples as a source of DNA increases the risk of genotyping errors. The low quality and quantity of DNA used as template in the polymerase chain reactions (PCR) might result in allelic dropout, *i.e.* one of the two alleles of a heterozygote may not be detected (Pemberton et al. 1995). Potential sources of genotyping errors also include scoring false alleles (PCR-generated alleles, Taberlet et al. 1996) or non-target fragments (Bradley & Vigilant 2002). Genotyping errors result in considering a "recapture" event as the "capture" of a new individual and thus, might bias the population size estimate. To limit the risk of genotyping error, the allele scoring should be based on several PCR replicates using, when feasible, different DNA extracts of the same sample as DNA template (Goossens et al. 2000; Navidi et al. 1992; Taberlet et al. 1996).

The capercaillie (*Tetrao urogallus* L.) is a grouse species with its main natural range in the taiga-like forests from Norway to Siberia. The species also occurs in the mountainous areas covered with coniferous uneven-aged forests in Western and Central Europe (Storch

2001). Changes in sylvicultural practices and increasing human disturbances have resulted in the fragmentation of its habitat in Scandinavia (Helle et al. 1994) and Central Europe, especially in lowland areas (Storch 2000, 2001). The first national survey of the capercaillie population in Switzerland was conducted by Glutz von Blotzheim *et al.* (1973), who compiled information collected during the years 1968–71 on the lek locations and number of males per leks by wildlife wardens, and assessed a minimum of 1100 capercaillie males in Switzerland. Those same leks as well as the newly established ones were monitored in 1985 by wildlife wardens and the results of this second national survey were published by Marti (Marti 1986), who estimated 550-650 males in Switzerland. The observed decline of 41-50% in male counts within 15 years was mostly attributed to lek extinctions (Marti 1986). During the latest national survey, conducted in 2001, 110 volunteers counted the number of males in 214 leks, at which the presence of an individual had been recorded since 1985 (Mollet et al. 2003). The authors extrapolated the number of males in areas not investigated by counting one additional male per unmonitored lek situated in areas in which sporadic direct observations of the species were reported. In those leks where the presence of the species was certain, these authors added the mean number of males per lek observed during the survey. In total, 450-500 males were estimated, thus providing further evidence of the decline of the capercaillie in Switzerland, both in population size and distribution since the national survey of 1985 (Mollet et al. 2003). An action plan was initiated to investigate the causes of the species decline and to determine the strategies for the maintenance of the species in Switzerland (Mollet in prep.).

We conducted this study to evaluate the performance of four rarefaction methods to estimate the census sizes of eleven local capercaillie populations in the Swiss Alps, and how they relate to field estimates. Selecting the most suitable estimation method will allow us to monitor the species dynamics in response to conservation efforts.

Material and methods

Sample collection and storage

The capercaillie inhabits large coniferous forests of mountainous areas in the Jura, the central and eastern Prealps and eastern Alps of Switzerland (Mollet et al. 2003). In the central Prealps, the capercaillie is distributed over large and continuous areas of suitable forest. In the eastern Prealps, it persists in isolated forest patches surrounded by agricultural landscapes. The situation is intermediate in the Central Alps, where patches of suitable habitat are surrounded by a matrix of suboptimal forest along the valley slopes. During the breeding

season, from late winter to mid spring, males and females aggregate around lekking arenas, usually situated in opened forest stands, clearings or fens.

Three study areas were investigated, between January and May, over a period of three years (2001-2003; Fig. 1). We focused our study in those forest where capercaillie presence had been reported in at least one of the national surveys of 1971 (Glutz von Blotzheim et al. 1973) and 1985 (Marti 1986). The procedures used to infer the presence of a *T. urogallus* population is described in details in Bollmann *et al.* (2005). In brief, we recorded direct (sightings) and indirect (mainly faeces, feathers, and footprints) evidences of capercaillie presence. Surveying was mostly focused on key elements of capercaillie spring and winter habitat.

Noninvasive samples for genetic analyses were collected during the field surveys. To maximise the number of individuals sampled, we selected feathers and faecal samples separated by a distance of at least 100 m. We distinguished among male and female samples, based on differences in the feather coloration and in the size and shape of the faecal samples. Faecal samples were collected and stored in 15-ml plastic tubes filled with approximately 5 ml of silica gel, or simply air-dried in the early stage of the study. Feathers were collected in paper envelopes or plastic bags (with or without silica gel). Samples were stored at room temperature or frozen at -20°C. We collected more than one thousand samples during the field surveys and analysed 530 of those (90% faecal samples and 10% feathers).

The distances among leks in the study area ranged between 2–3 km in suitable forests and up to 5–10 km in fragmented habitats. These distances were in the range of published studies on lek distribution in central Europe and Scandinavia (Picozzi et al. 1992; Rolstad & Wegge 1987; Storch 1995). Thus, samples separated by less than 2 km distance were grouped into 26 discrete units (local populations). We conducted capture-recapture estimations of the census sizes in those eleven local populations, in which the number of samples collected was largest.

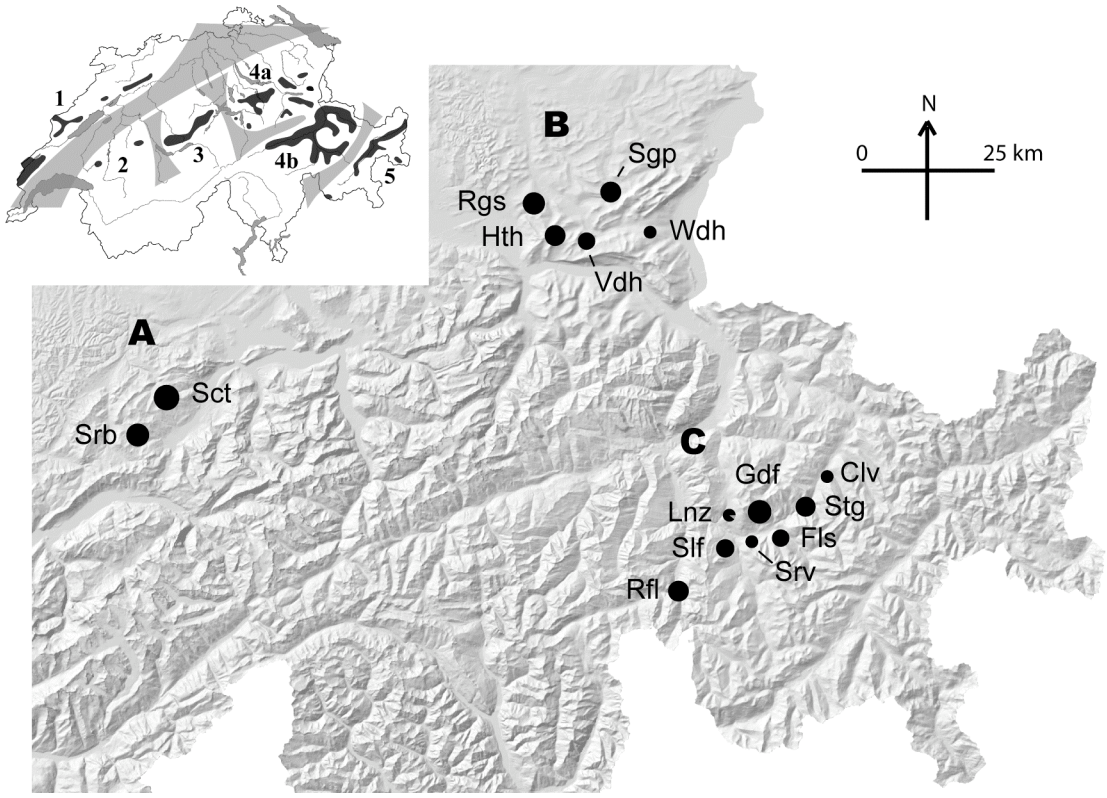


Figure 1*: Location of the fifteen local populations investigated and their division into the study areas A-C. The plot areas are proportional to minimum number alive* (MNA). *We estimated the census sizes of the eleven largest local populations.* MNA *was used as an approximation of the census sizes in the four smallest populations. Abbreviations and full name of the populations are listed in Table 2.* Inset*: Distribution of* Tetrao urogallus *in Switzerland (dark shading). Putative barriers to dispersal among the five regions (1-5) are indicated in light shading (map from Mollet et al. 2003).*

DNA extraction and genotyping

We extracted DNA in a dedicated room and using filtered tips to avoid carry-over contaminations by PCR products and cross-contaminations. We included negative extraction controls to monitor contaminations.

DNA from faecal samples was extracted using the DNA Stool MiniKit (Qiagen, Hombrechtikon) following an adapted protocol. A fragment of each dropping (0.2 to 0.5 g) was incubated at room temperature for 12 h in 3 ml buffer ASL and the extraction columns were washed twice with 400 μL of buffer AW1. The DNA was eluted in 2 x 75 μl buffer AE.

DNA from feathers was extracted using the QIAmp Tissue extraction kit (Qiagen, Hombrechtikon) following the protocol provided by the manufacturer. The tip of a feather (0.5 to 1 cm) was cut into small pieces and incubated overnight at 37°C with proteinase K. The DNA was eluted in 2 x 75 μL of buffer AE.

To amplify fragments in the range of 50–150bp, we designed new primer pairs, based on the sequences available in Genbank, for ten microsatellite loci developed for the capercaillie (Segelbacher et al. 2000). We amplified two additional microsatellite loci, BG15 and BG18, developed for *Tetrao tetrix* L. (Black grouse, Piertney & Höglund 2001). We distinguished between the three grouse species present in the study area, *T. urogallus*, *T. tetrix* and *Bonasa bonasia* L. (hazel grouse), based on different allele size ranges at those two loci (Table 1). The twelve microsatellite loci were amplified in four multiplex-PCRs, each containing three primer pairs differing in their fluorescent labelling dyes (FAM, HEX, NED; Applied Biosystems, Rotkreuz). We amplified a nuclear gene fragment to ascertain the sex of the individuals (Griffiths et al. 1998). PCRs were set up in 10 μl volumes containing 1 μl of DNA extract, 1x Multiplex Kit MasterMix (Qiagen, Hombrechtikon), 1 mM MgCl2, 0.1 μg/μl BSA and 160 nM of each primer. The amplification was done on a PT-100 thermocycler (MJResearch, Bioconcept, Allschwil) with the following steps: an initial polymerase activation (HotStart PCR) at 95°C for 15 min, 37 cycles of 94°C for 30s, 56°C (microsatellites) and 46°C (sex identification) for 120s, 72°C for 30s, and a final extension at 72°C for 45 min. Negative controls for extraction and PCR were included. We amplified a reference sample as a positive control and to control that the electrophoretic mobility of the fragments was consistent among runs (Davison & Chiba 2003). The amplification products were visualized on an ABI3100-Avant automated sequencer (Applied Biosystems, Rotkreuz). The allele lengths were coded using GENESCAN® 3.1 and GENOTYPER® 2.5 (Applied Biosystems, Rotkreuz), relative to an internal size standard (ROX 400HD, Applied Biosystems, Rotkreuz). We visualized the products of the sexing PCR on 3% agarose gels to determine the sex of the individuals, as recommended by Griffiths et al. (1998).

Table 1*: Characteristics of the twelve nuclear microsatellite markers used in this study. We redesigned ten primer pairs (designated with the prefix "s"), based on the sequences available on Genbank (see Material and Methods for details).* A_o *refers to the number of alleles detected in the capercaillie. The allele size ranges are indicated for the three grouse species present in the study area:* Tetrao urogallus *(capercaillie,* Tu*),* T. tetrix *(black grouse,* Tt*) and* Bonasa bonasia *(hazel grouse,* Bb*).*

Locus	Primer sequences (5'-3') or reference	Genbank accession number	Multiplex group	A_o	Size range		
					Tu	*Tt*	*Bb*
sTuD1	F:ATTTGCCAGGAAACTTGCTC	AF254644	1	7	149-163	153-161	149
	R:CCTTTGCCTCCTTATGAAATCC						
sTuD7	F:GGGTCATTAGGCAGAGCTTTC	AF254650	1	4	94-100	92-98	100-106
	R:CCTGCATCATTCCAAATGTC						
sTuT4	F:TGGGAGCATCTCCCAGAGTC	AF254656	1	6	122-142	118-146	92-94
	R:ACAAACAAGGCAGCAGCATG						
BG15	from Piertney and Höglund (2001)		2	5	130-146	179-191	127-131
sTuD3	F:CAAGGGGAAAATATGTGTGTG	AF254646	2	10	61-99	77-91	83
	R:TGTCAAGATATTTCAAGCCTTTG						
sTuT1	F:TGTATATCTGTCTGTCTGCCCGTC	AF254653	2	8	100-140	132	NA
	R:GCACAGGAACAGCAATAGATGG						
sTuD6	F:AGCCTTTTACTGCACTACTTGC	AF254649	3	12	160-192	146-154	160-162
	R:GGTGTGTGGGAAATGAGGAC						
sTuT3	F:GCCTCAACTAATCACCCCTTTATC	AF254655	3	7	81-109	93-109	143-159
	R:GAGGGATTTATGCATGCTGCTAG						
sTuD5	F:GGCTGTACACAGCACTGAGC	AF254648	3	13	121-153	89	87
	R:GGGATGCAGCTGTGATAGTG						
sTuT2	F:TCTCCAAACTAGATATGGAAACCAG	AF254654	4	8	143-199	119-123	155-193
	R:CAAAGCTGTGTTTCATTAGTTGAAG						
sTuD4	F:TGCACATACATAACATGCAGCC	AF254647	4	12	52-90	80	NA
	R:TGGGAGGACTGTGTAGGAGAGC						
BG18	from Piertney and Höglund (2001)		4	7	186-210	141-170	130-152
P2	from Griffiths *et al.* (1998)						
P8	from Griffiths *et al.* (1998)						

We followed the multitube approach, as recommended by Navidi et al. (1992) and Taberlet et al. (1996) and amplified each DNA extract in four reactions. We first amplified BG15, TuD3 and TuT1 (Multiplex n°1) to assign the samples to one of the three grouse species present in the study area. The genotype at each locus was recorded if the same allele combination was observed in three or more PCR replicates and left blank otherwise. Samples with one or two missing loci were amplified in four additional PCR replicates and their genotypes was recorded if the same allelic combination was observed in three out of eight replicates. Loci that could not be scored after eight PCR replicates were coded as missing

values. Samples with a low prospect of producing a multi-locus genotype (no amplification products at any of the three loci) and those assigned to black grouse or hazel grouse were discarded. Capercaillie samples were typed with the nine remaining microsatellite markers, organised in three multiplexes, and the sex-specific locus, following the same genotyping procedure.

Data analysis

We first built an input file restricted to unique genotypes, using the grouping function implemented in the program GIMLET (Valière 2002). We then computed the probability of two individuals in the population sharing the same genotype using the formula proposed by Paetkau and Strobeck (1994) and from Taberlet and Luikart (1999). The latter formula accounts for the sampling of relatives and provides the most conservative measurement, noted PI_{sib}, which provided us with an upper range for the probability of identity among individuals. We considered the risk of two individuals sharing the same genotype to be negligible if PI_{sib} values were below 0.01, as recommended for estimations of population census sizes (Waits et al. 2001). We calculated exact tests of Hardy–Weinberg equilibrium (HWE) for each population using the program GENEPOP 3.3 (Raymond & Rousset 1995) and the algorithm of Guo and Thompson (1992). We computed the associated P-values with a Markov Chain procedure and the default parameters for the dememorisation steps (10000), the number of batches (100) and the number of iterations per batch (5000) as implemented in GENEPOP 3.3.

Because the grouping of genotypes with missing values might be ambiguous, the program GIMLET uses only complete genotypes to estimate population sizes (N. Valière, University of Lyon, pers. comm.). However, some genotypes in our data set were incomplete and, thus, would not have been included in the estimations, despite showing a unique allelic combination in those loci that were amplified. We therefore grouped the allelic combinations into unique genotypes (not considering those loci that were not amplified) and coded those with consecutive numbers. In each local population, we assessed the number of unique genotypes, reported as the minimum number of individuals alive (MNA). We then estimated the census sizes of the local populations with those three models implemented in GIMLET (Valière 2002). The number of unique genotypes (y) was expressed as a function of the number of samples analysed (x). The asymptotic value (a) of the fitted curve provided an estimate of the population census size. Kohn *et al.* (1999) estimated the census size of a

coyote (*Canis latrans*) population using the hyperbolic function: $y = ax/(b+x)$, referred to as Kohn's model, where b was the non-linear slope of the function. As the shape of the accumulation curve depended of the order in which the samples are added (Kohn et al. 1999), these authors averaged the estimates of a over 1000 iterations and built a 95% confidence interval. Eggert *et al.* (2003) estimated the density of a forest elephant (*Loxodonta cyclotis*) population using the exponential function: $y = a\ (1\text{-}e^{(bx)})$, referred to as Eggert's model, b as above. Valière (2002) reported a third function: $y = a\text{-}a(1\text{-}(1/a))^x$, referred to as Chessel's model, which Wilson *et al.* (2003) used to estimate the census size of a badger (*Meles meles*) population. This function modelled the expectancy of the number y of boxes (individuals) filled, when x balls (faecal samples) are distributed. GIMLET (Valière 2002) automatically generated a script to calculate the asymptotic value of Chessel's, Eggert's and Kohn's models using the statistical package R (R Development Core Team 2005). We also used a program developed to estimate the census size of small populations, based on an urn model with replacement (Capwire, Miller et al. 2005). Two models were implemented in Capwire to account for equal frequencies of capture among individuals (the even capture model, ECM) or for different frequencies of capture among individuals (two innate rates model, TIRM). We therefore tested if the frequency of capture differed among individuals in each local population, using a Wilcoxon one-sample test, to decide for which of those two models was most appropriate to analyse our data.

Results

Multi-locus genotyping and population analysis

We could amplify one or more loci from 351 DNA extracts (66%), of which 279 were assigned to *T. urogallus*, 28 to *T. tetrix* and eight to *B. Bonasia* based on the species-specific allele size ranges observed at BG15 and BG18. Five genotypes showed allele sizes specific for both capercaillie and black grouse and thus indicated hybridization events between *T. urogallus* and *T. tetrix*. Thirty-one samples did not amplify at those two loci. Of the 279 capercaillie samples, 244 samples successfully amplified at eight to twelve loci. Those 244 multilocus genotypes grouped into 151 unique allelic combinations at those loci that were amplified. We restricted our data set to those 15 local populations situated in three study areas (Fig. 1, Table 2). Minimum number alive *(MNA)* was two in Wildhaus, Lenz, Clavadel and Surava. Thus, we used *MNA* as an approximation of the census sizes in those local populations and did not report their genetic characteristics (Table 2). In the eleven remaining

local populations, *MNA* ranged from six in Filisur and Vorderi Höhi to 17 in Schlierental. None of the individuals were observed in more than one local population, and, thus, we assumed the local populations to be closed to migration during our study. The percentage of samples successfully genotyped per local population (considering *T.urogallus*, *T.tetrix* and *B.bonasia* samples), ranged from 45% in Hinteri Höhi, to 86% in Filisur and Vorderi Höhi (mean = 66%; Table 2).

***Table 2**: Genotyping success and characteristics of the eleven local populations investigated. We report the overall sample size* N *and percentage of successfully amplified samples* $\%_{Success}$*, including capercaillie, black grouse and hazel grouse. For the samples assigned to capercaillie, we report the number of males (*M*), the number of females (*F*) and the number of individuals for which the sex could not be identified (?). In each local population, we calculated the overall mean frequency of capture,* MFC*, the mean frequency of capture per male,* MFC_m*, and per female,* MFC_f*. We calculated the index of dispersion,* I_{Disp}*, as the ratio* VFC/MFC*. We estimated the probability of two individuals having the same genotype assuming random mating,* PI*, and the probability of two siblings having the same genotype,* PI_{sib}.

				Capercaillies								
Populations	Abb.	*N*	$\%_{Success}$	*M*	*F*	*?*	*MFC*	MFC_m	MFC_f	I_{Disp}	*PI*	PI_{sib}
Sörenberg	Srb	16	81.3	6	7	-	1.23[b]	1.33	1.14	0.36	9.7×10^{-8}	8.7×10^{-4}
Schlierental	Slt	27	63.0	10	7	-	1.59[b]	1.60	1.57	0.88	3.1×10^{-8}	5.3×10^{-4}
Regelstein	Rgs	24	50.0	6	5	1	1.85[b]	1.83	2.00	1.97	6.3×10^{-9}	3.0×10^{-4}
Hinteri Höhi	Hth	22	45.5	3	5	2	2.20[b]	2.00	2.00	0.40	6.4×10^{-9}	3.0×10^{-4}
Vorderi Höhi	Vdh	7	85.7	4	2	-	1.17[b]	1.25	1.00	0.17	3.0×10^{-7}	1.2×10^{-3}
Schwägalp	Sgp	17	58.8	5	4	1	1.70[b]	2.00	1.50	1.12	4.3×10^{-9}	2.6×10^{-4}
Wildhaus[a]	Whs											
Rofla	Rfl	18	55.6	5	5	-	1.80[b]	1.80	1.80	2.18	6.4×10^{-7}	1.6×10^{-3}
Lenz[a]	Lnz											
Got Dafora	Gdf	20	70.0	9	5	-	1.43[b]	1.44	1.40	0.42	1.9×10^{-10}	9.0×10^{-5}
Steigwald	Stg	14	75.0	5	4	-	1.33[b]	1.40	1.25	0.50	2.8×10^{-9}	2.0×10^{-4}
Clavadel[a]	Clv											
Filisur	Fls	7	85.7	3	2	1	1.17[b]	1.33	1.00	0.17	1.6×10^{-9}	1.6×10^{-4}
Surava[a]	Srv											
Salouf	Slf	12	58.3	6	1	-	1.71[b]	1.83	1.00	0.90	3.8×10^{-8}	4.7×10^{-4}

[a] no census sizes estimates were calculated for those local populations, we therefore did not describe their characteristics

[b] no significant differences among individuals in the frequency of capture

Population genetic characteristics

Five local populations deviated from HWE and showed a deficit of heterozygote individuals. Over all local populations, three loci (TuD4, TuD6 and TuT1) deviated from HWE (data not shown). The probability of two unrelated individuals sharing the same genotype were low. The *PI* ranged from 1.9 x 10-10, in Got Dafora, to 6.4 x 10-7 in Rofla. The same pattern was observed for the probability of two siblings sharing the same genotype with PI_{sib} ranging from 9.0 x 10-5 in Got Dafora to 1.6 x 10-3 in Rofla.

Field census and minimum number alive

There was no bias in the sex ratio averaged over all the eleven local populations (Wilcoxon 1-sample, p>0.5) and we could show that the probability of finding droppings or feathers of males was larger than for females (Wilcoxon 1-sample, p=0.032). We therefore assumed a sex ratio of 1:1 to extrapolate to the census sizes of the eleven local populations from the number of males estimated in the field survey (Table 2). The field census estimates were larger than the number of unique genotypes observed in four local populations, Sörenberg, Schwägalp, Got Dafora and Salouf. In three local populations, Schlierental, Rofla and Filisur, the range of the field estimates covered the MNA. In the four remaining local populations, the field survey underestimated the number of individuals; 2-4 individuals each were estimated in Regelstein, Hinteri Höhi and Vorderi Höhi, and twelve, ten and six unique genotypes were identified in those local populations, respectively. In Steigwald, we identified nine unique genotypes, where 4–6 individuals were estimated.

Rarefaction analyses

There was no differences in the frequency of capture among individuals in the eleven local populations investigated (Wilcoxon rank test, p>0.5), and, thus, we estimated the census sizes of those local populations using ECM, as implemented in Capwire (Table 2).

We separated the populations by their sample size, *N*, and index of dispersion I_{Disp}, calculated as the ratio *VFC*/*MFC*, where *VFC* is the variance in the frequencies of capture per individual and *MFC* is the mean frequency of capture per individual (Fig. 2). We applied this classification with the aim to ease the comparison among the four models in the eleven local populations.

Kohn's model consistently estimated largest census sizes (up to 300% larger than the three other models) and showed largest confidence interval (*CI*). These results suggested that Kohn's model overestimated the census sizes in those local populations. We therefore do not report the census sizes and *CI* estimated with Kohn's model.

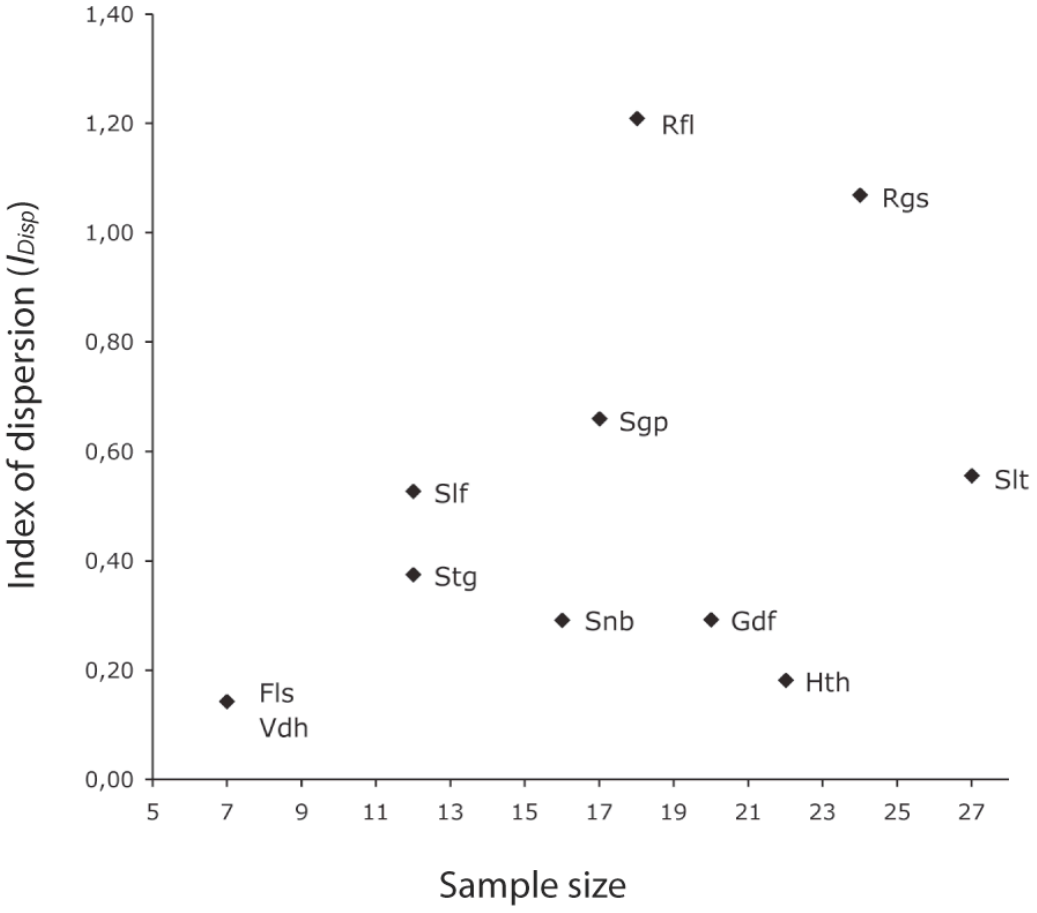


Figure 2*: Scatter plot of the eleven study populations separated by their sample size analysed,* N, *and index of dispersion,* I_{Disp}. *Abbreviations and full name of the populations are listed in Table 2.*

Filisur and Vorderi Höhi had the smallest *VFC*, *MFC*, sample sizes and *MNA*. Eggert's model and ECM provided similar estimates (17–19 individuals) and ECM showed the narrowest *CI*. Chessel's model estimated census sizes of 23 individuals and showed large *CI*. Hinteri Höhi was characterized by the largest MFC and the third largest sample size (*N*=22). Chessel's and Eggert's model and ECM estimated 11–12 individuals, and Chessel's

model and ECM showed the narrowest *CI*. Steigwald, Sörenberg, Got Dafora and Schlierental were characterized by median value of *VFC* (0.36–0.88) and *MFC* (1.23–1.59). Chessel's model and ECM provided similar estimates, and Chessel's model showed narrower *CI* than ECM. Eggert's model estimated larger census sizes than Chessel's model and ECM, but the difference among the model was low in Steigwald (*N*=12). In Salouf, the distribution of the frequencies of capture per individuals was bimodal. Chessel's and Eggert's model and ECM estimated 7–9 individuals, and Eggert's model showed the largest *CI*. The *CI* of Chessel's model and ECM overlapped. Regelstein, Schwägalp and Rofla were characterized by large *VFC* (>1.12) and *MFC* (>1.80). Chessel's model and ECM estimated similar census sizes and ECM had the narrowest *CI*. Eggert's model provided estimates and *CI* larger than Chessel's model and ECM.

Our results suggest that Chessel's model and ECM are best to estimate the census sizes of local populations. Thus, we estimated the number of individuals in the three study areas from the results of Chessel's model and ECM (Fig. 1). For this purpose, we summed up the census sizes estimated in local populations, and *MNA* if no census size estimate was conducted (Table 3). In the study area A, Chessel's model estimated 62 individuals (39–114), and ECM 61 individuals (31–146), that is 50% more than the field estimates of 44 individuals (36–56). In the study area B, 67 (54–97, Chessel's model) and 61 (41–77) individuals were estimated from capture–recapture, that is 120% more than the field estimates of 28 (22–32) individuals. In the study area C, Chessel's model estimated 93 (58–147) individuals and ECM 90 (52–181), that is 40% more than the field estimates of 64 (56–82).

Discussion

Data collection and suitability of the markers

In this study, we used non-invasive samples as source of DNA and a multiplex-PCR approach to estimate the census sizes of eleven local populations of *T. urogallus* in the Swiss Alps. We established a strict genotyping procedure to limit the risk of cross-contaminations and genotyping errors, which might arise when working with degraded DNA. Working with noninvasive samples demands large human and financial resources, which might confine those methods to the studies of rare or endangered species, despite of their advantages (no physical capture of individuals, low disturbance of the social structures of the investigated population). However, recent improvements in the sample collection and storage procedures (Frantzen et al. 1998; Murphy et al. 2002; Nsubuga et al. 2004), and DNA extraction and

amplification methods (Horvath et al. 2005; Piggott et al. 2004) might contribute to the generalization of the non-invasive sampling techniques in population genetic studies.

***Table 3**: Census sizes and confidence interval in the eleven local populations. For each local population, we indicate the region and study area of origin. We report the number of males,* N_m*, the associated confidence interval,* CI_m*, and the confidence interval of local populations census sizes,* CI_{pop}*, estimated from field surveys. We enumerated the number of unique genotypes,* i.e. *minimum number of individuals alive,* MNA. *For Chessel's, Eggert's and Kohn's models, we report* a*, the census size estimated based on 1000 iterations and the 95% confidence interval,* CI. *ECM provide an estimate of the census size noted* N*, and the confidence interval around this estimate,* CI.

Study area		Field survey			Chessel's model		Eggert's model		Kohn's model		Capwire	
(Region)	Pop	N_m (CI_m)	CI_{pop}	*MNA*	*a*	*CI*	*a*	*CI*	*a*	*CI*	*N*	*CI*
A (3)	Srb	12 (10–15)	20–30	13	37	21–76	49	20–792	127	34–5065	35	14–115
	Slt	10 (8–13)	16–26	17	25	18–38	34	18–552	59	27–1042	26	17–41
	Total		36–56		62	39–114					61	41–156
B (4a)	Rgs	2 (1–2)	2–4	12	16	11–22	26	12–518	47	16–1729	16	13–22
	Hth	2 (1–2)	2–4	10	12	10–15	12	9–28	17	12–51	11	10–13
	Vdh	2 (1–2)	2–4	6	23	13–38	18	12–35	32	21–65	19	6–19
	Sgp	6 (6–7)	12–14	10	14	8–20	19	10–454	35	14–1444	13	10–21
	Whs	2 (2–3)	4–6	2	2[a]	2[a]					2[a]	2[a]
	Total		22–32		67	44–97					61	41–77
C (4b)	Rfl	4 (4–5)	8–10	10	12	8–18	18	10–215	30	13–428	13	10–19
	Lnz	2 (2–4)	4–8	2	2[a]	2[a]					2[a]	2[a]
	Gdf	12 (10–17)	20–34	14	26	16–43	32	16–259	62	25–1887	25	14–57
	Stg	3 (2–3)	4–6	9	19	11–33	23	12–227	50	19–1265	18	9–62
	Clv	2 (1–2)	2–4	2	2[a]	2[a]					2[a]	2[a]
	Fls	2 (2–3)	4–6	6	23	13–38	17	12–35	31	21–65	19	6–19
	Srv	2 (2)	4	2	2[a]	2[a]					2[a]	2[a]
	Slf	5 (5–6)	10–12	7	7	4–9	9	5–140	14	7–277	9	7–18
	Total		56–84		93	58–147					90	52–180

[a] MNA was used as an approximation of the census size

TuD6 and TuT1 deviated from HWE and showed a large proportion of missing values and a deficit in heterozygous individuals in three neighbouring local populations. This suggested that the presence of one or more null alleles at TuD6 and TuT1 in those three local populations, rather than a high rate of allelic dropout, explained the observed deviation from

HWE. We kept those loci, as they were the 4th (PI_{TuD6} = 0.110) and the 6th (PI_{TuT1} = 0.184) most informative loci overall to discriminate among individuals.

Two alleles at least differed among all observed allelic combinations, which indicated that our set of markers was powerful enough to discriminate among individuals and, thus, was suitable to investigate the census sizes in capercaillie local populations.

Census size estimators

In our study, we could show that Kohn's model tended to overestimate the census sizes of most local populations and showed large CI. These results supported the simulations studies of Petit and Valière (Petit & Valière in press) who showed that this model overestimated the census sizes and was characterized by the largest variance for all sampling intensities and populations sizes. Eggert's model estimated census sizes in the range of those from Chessel's model and ECM in those local populations characterized by small sample sizes or large *MFC*, but consistently showed large *CI*. Those conclusions were suggested by Miller et al. (2005) and Petit and Valière (Petit & Valière in press). We therefore suggest that Eggert's and Kohn's models were not suitable to estimate the census sizes of small populations. In fact, Eggert's and Kohn's model estimate an asymptotic value for the number of individuals, and, thus, are likely to overestimate the true population size.

In contrast, Chessel's model and ECM assume finite values for the number of individuals and, thus, provide estimates closest to the real census sizes. However, no comparison of the performance of Chessel's model and ECM have been published so far, and we lack empirical data to decide which of these two models is appropriate to analyse our data. Chessel's model and ECM provided close census size estimates in the eleven local populations. Chessel's model tended to estimate larger census sizes than ECM in those local populations characterized by a low sample size (Vorderi Höhi and Filisur) or by a bimodal distribution of the frequency of capture per individual (Salouf). Chessel's model tended to show narrowest *CI* in these populations characterized by low values of *VFC* and *MFC*. Thus, we suggest that Chessel's model and ECM are suitable to estimate the census sizes of small populations.

In Schlierental, Schwägalp and Got Dafora, the capture–recapture estimates were close to those from the field survey. Schlierental and Schwägalp were situated in habitats considered as typical for the capercaillie in the Swiss Prealps, *i.e.* coniferous and mixed deciduous forests dominated by *Picea abies* (L.) Karst (Norway spruce) and *Fagus sylvatica*

L. (Common beech) inter-connected by large mires (Schlierental). Got Dafora and Salouf represent habitat considered as typical for the capercaillie in the Swiss Alps, *i.e.* forests mainly dominated by the conifers *P. abies* and *Pinus mugo* Turra (Mountain pine) with *Pinus sylvestris* L. (Scots pine) occurring on the south-exposed slopes and dense *Vaccinium myrtillus* L. (Bilberry) cover in the understorey. In Sörenberg, Rofla, Steigwald and Filisur, the capture-recapture methods provided census size estimates larger than those from the field survey. The field census in these four local populations was complicated by the uneven topography encountered (Sörenberg) or by the presence of distinct forest patches (Filisur). Rofla and Steigwald were isolated, and this might have resulted in population densities higher than expected (Bowers & Matter 1997; Bowman et al. 2002). Finally, we could show that the capture-recapture method outperformed the field method in estimating the census sizes of Regelstein, Hinteri Höhi and Vorderi Höhi. These three local populations were situated in what was recognized as good pre-alpine capercaillie habitats. However, the distribution pattern of the capercaillie and the location of the lekking sites in those local populations were poorly known, which might have resulted in the underestimation of the number of males.

Thus, we could show that the field census was a good approximation and thus provided a fast and cost-effective method to estimate the sizes of well-known local populations. However, estimating the census sizes from field evidences might result in the underestimation of the census sizes of the local populations where the habitat is not typical or where the distribution of the leks is not known. Genetic capture–recapture estimates appear to be less influenced by the degree of knowledge of the study area. We therefore recommend to estimate the census sizes of populations living in unusual habitats, such as ecological traps (Battin 2004) from noninvasive samples and capture–recapture analysis. The choice of the model to estimate population census sizes depends on the sampling intensity. Based on our results, we recommend to estimate the census sizes of small populations using Chessel's model (*VFC* and *MFC*<1) or ECM (*VFC* and *MFC*>1 and very small populations).

Recommendations for the monitoring program of the capercaillie in Switzerland

In this study, we compared the census size estimates in eleven local populations divided into three core areas of the distribution of the capercaillie in the Swiss Alps. We could show that the prior knowledge of the capercaillie distribution and habitat requirements positively influenced the accuracy of field estimates. Thus, a better knowledge of the habitat

characteristics that explain capercaillie numbers in local populations are necessary if the species monitoring program should rely solely on field surveys. Recently, Graf et al. (2004; 2005) showed that the set of ecological variables explaining the presence of the capercaillie differed between the Swiss Prealps and the Alps, which might improve our knowledge on the species habitat requirements at the landscape level. Bollmann et al. (2005) characterized the capercaillie habitat in the central Swiss Alps, which might help improving the estimation of the census sizes of local populations in those habitats. However, little is known on the habitat requirements of the females, and direct observations of females are seldom counted during field surveys (Glutz von Blotzheim et al. 1973; Marti 1986; Mollet et al. 2003). Thus, field surveys based on direct and indirect evidences from both sexes would improve the estimations of the local populations census sizes. More studies are necessary to determine which ecological factors influence the densities of capercaillie in the Swiss Alps and Prealps.

We could show, in this study, that genetic capture–recapture estimates are suitable to estimate the census sizes of small populations, and might be appropriate for the monitoring of capercaillie populations. However, this method might not yet be applicable to investigate large areas of the species distribution because of the cost associated with individual genetic-tagging. Therefore, we recommend to perform genetic capture–recapture studies in focal populations to calibrate field estimates and thus achieve accurate estimates of census sizes of capercaillie populations in Switzerland. These methods will allow us to monitor the response of the capercaillie to future management actions, such as habitat quality improvement (Mollet in prep.). We will also be able to evaluate the strategies to maintain the capercaillie in Switzerland, which will be planned according to ongoing investigations of the genetic structure among local populations.

Our approach could be applied to estimate the census sizes of a wide range of species and is recommended to investigate secretive species and species living in habitats not easily accessible.

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References

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos*, 71, 355-366.

Banks SC, Hoyle SD, Horsup A, Sunnucks P, Taylor AC (2003) Demographic monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorhinus krefftii*) by genetic analysis of non-invasively collected material. *Anim. Conserv.*, 6, 101-107.

Bascompte J, Solé RV (1996) Habitat fragmentation and extinction thresholds in spatially explicit models. *J. Anim. Ecol.*, 65, 465-473.

Battin J (2004) When good animals love bad habitat: ecological traps and the conservation of animal populations. *Conservation Biology*, 18, 1482-1491.

Bollmann K, Weibel P, Graf RF (2005) An analysis of central Alpine capercaillie spring habitat at the forest stand scale. *Forest Ecology and Management*, 215, 307-318.

Bowers MA, Matter SF (1997) Landscape ecology of mammals: Relationships between density and patch size. *J. Mammal.*, 78, 999-1013.

Bowman J, Cappuccino N, Fahrig L (2002) Patch size and population density: the effect of immigration behavior. *Conservation Ecology*, 6.

Bradley BJ, Vigilant L (2002) False alleles derived from microbial DNA pose a potential source of error in microsatellite genotyping of DNA from faeces. *Mol. Ecol. Notes*, 2, 602-605.

Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. *Phil. Trans. R. Soc. Lond. B*, 345, 101-118.

Davison A, Chiba S (2003) Laboratory temperature variation is a previously unrecognized source of genotyping error during capillary electrophoresis. *Mol. Ecol. Notes*, 3, 321-323.

Eggert LS, Eggert JA, Woodruff DS (2003) Estimating population size for elusive animals: the forest elephant of Kakum National Park, Ghana. *Mol. Ecol.*, 12, 1389-1402.

Fisher DO, Hoyle SD, Blomberg SP (2000) Population dynamics and survival of an endangered wallaby: A comparison of four methods. *Ecological Applications*, 10, 901-910.

Frantz AC, Schaul M, Pope LC, Fack F, Schley L, Muller CP, Roper TJ (2004) Estimating population size by genotyping remotely plucked hair: the Eurasian badger. *Journal of Applied Ecology*, 41, 985-995.

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH (1998) Empirical evaluation of preservation methods for faecal DNA. *Mol. Ecol.*, 7, 1423-1428.

Franzreb KE (1997) Success of intensive management of a critically imperiled population of Red-cockaded Woodpeckers in South Carolina. *J. Field Ornithol.*, 68, 458-470.

Fujiwara M, Caswell H (2001) Demography of the endangered North Atlantic right whale. *Nature*, 414, 537-541.

Gagneux P, Boesch C, Woodruff DS (1997) Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Mol. Ecol.*, 6, 861-868.

Gerloff U, Schlötterer C, Rassmann K, Rambold I, Hohmann G, Fruth B, Tautz D (1995) Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living Bonobos (*Pan paniscus*). *Mol. Ecol.*, 4, 515-518.

Glutz von Blotzheim UN, Bauer KM, Bezzel E (1973) *Tetrao urogallus*. In: *Handuch der Vögel Mitteleuropas* (ed. Verlagsgesellschaft A), pp. 172-225, Frankfurt am Main.

Goossens B, Chikhi L, Utami SS, de Ruiter J, Brudford MW (2000) A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. *Conservation Genetics*, 1, 157-162.

Graf RF, Bollmann K, Suter W, Bugmann H (2004) Using a multi-scale model for identifying priority areas in capercaillie (*Tetrao urogallus*) conservation. In: *Proceedings of the 12th annual IALE(UK) conference "Landscape ecology of trees and forest"* (ed. Smithers R), pp. 84-90, Cirencester, UK.

Graf RF, Bollmann K, Suter W, Bugmann H (2005) The importance of spatial scale in habitats model: capercaillie in the Swiss Alps. *Landscape Ecology*, 20, 703-717.

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. *Mol. Ecol.*, 7, 1071-1075.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48, 361-372.

Helle P, Helle T, Lindén H (1994) Capercaillie (*Tetrao urogallus*) lekking sites in fragmented Finnish forest landscape. *Scand. J. For. Res.*, 9, 386-396.

Horvath MB, Martinez-Cruz B, Negro JJ, Kalmar L, Godoy JA (2005) An overlooked DNA source for non-invasive genetic analysis in birds. *J. Avian Biol.*, 36, 84-88.

Kohn MH, York EC, Kamradt DA, Haugt G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. *Proc. R. Soc. London Ser. B*, 266, 657-663.

Kreeger TJ, White PJ, Seal US, Tester JR (1990) Pathological responses of Red foxes to foothold traps. *Journal Of Wildlife Management*, 54, 147-160.

Lande R (1993) Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.*, 142, 911-927.

Marti C (1986) Verbreitug und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz. *Ornithol. Beob.*, 83, 67-70.

Maschinski J, Frye R, Rutman S (1997) Demography and population viability of an endangered plant species before and after protection from trampling. *Conservation Biology*, 11, 990-999.

Miller CR, Joyce P, Waits LP (2005) A new method for estimating the size of small populations from genetic mark-recapture data. *Mol. Ecol.*, 14, 1991-2005.

Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications*, 10, 283-294.

Mollet P (in prep.) Aktionsplan Auerhuhn 2005 – 2020. Schweizerische Vogelwarte, Sempach.

Mollet P, Badilatti B, Bollmann K, Graf RF, Hess R, Jenny H, Mulhauser B, Perrenoud A, Rudmann F, Sachot S, Studer J (2003) Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. *Ornithol. Beob.*, 100, 67-86.

Morin PA, Wallis J, Moore JJ, Chakraborty R, Woodruff DS (1993) Noninvasive sampling and DNA amplification for paternity exclusion, community structure, and phylogeography in wild chimpanzees. *Primates*, 34, 347-356.

Murphy MA, Waits LP, Kendall KC, Wasser SK, J.A. H, Bogden R (2002) An evaluation of long term preservation methods for brown bear (*Ursus arctos*) faecal DNA samples. *Conservation Genetics*, 3, 435-440.

Navidi W, Arnheim N, Waterman M (1992) A multiple-tubes approach for accurate genotyping of very small DNA samples using PCR: statistical considerations. *American Journal of Human Genetics*, 50, 347-359.

Nsubuga AM, Robbins MM, Roeder AD, Morin PA, Boesch C, Vigilant L (2004) Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Mol. Ecol.*, 13, 2089-2094.

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.*, 3, 489-495.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci — a caution for parentage and population studies. *Mol. Ecol.*, 4, 249-252.

Petit E, Valière N (in press) Estimating population size: emphasis on noninvasive CMR. *Conservation Biology*.

Picozzi N, Catt DC, Moss R (1992) Evaluation of Capercaillie Habitat. *Journal of Applied Ecology*, 29, 751-762.

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Mol. Ecol. Notes*, 1, 303-304.

Piggott MP, Bellemain E, Taberlet P, Taylor AC (2004) A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conservation Genetics*, 5, 417-420.

Pine WE, Pollock KH, Hightower JE, Kwak TJ, Rice JA (2003) A review of tagging methods for estimating fish population size and components of mortality. *Fisheries*, 28, 10-23.

R Development Core Team (2005) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.*, 86, 248-249.

Rolstad J, Wegge P (1987) Distribution and size of capercaillie leks in relation to old forest fragmentation. *Oecologia*, 72, 389-394.

Schwarz CJ, Seber GAF (1999) Estimating animal abundance: Review III. *Stat. Sci.*, 14, 427-456.

Segelbacher G, Paxton RJ, Steinbruck G, Trontelj P, Storch I (2000) Characterization of microsatellites in capercaillie *Tetrao urogallus*. *Mol. Ecol.*, 9, 1934-1935.

Storch I (1995) Annual home ranges and spacing patterns of Capercaillie in Central Europe. *Journal of Wildlife Management*, 59, 392-400.

Storch I (2000) Grouse Status Survey and Conservation Action Plan 2000-2004. (ed. Group WBSGS), p. 112 pp. IUCN, Gland, Switzerland and Cambridge, UK and the World Pheasant Association, Reading, UK.

Storch I (2001) *Tetrao urogallus* Capercaillie. *BWP Update*, 3, 1-24.

Taberlet P, Griffin S, Goosens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J (1996) Reliable genotyping of samples with very low DNA quantity using PCR. *Nucleic Acids Research*, 24, 3189-3194.

Taberlet P, Luikart G (1999) Non-invasive genetic sampling and individual identification. *Biol. J. Linnean Soc.*, 68, 41-55.

Travis JMJ (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proc. R. Soc. Lond. B*, 270, 467-473.

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes*, 2, 377-379.

Vanballenberghe V (1984) Injuries to wolves sustained during live-capture. *Journal Of Wildlife Management*, 48, 1425-1429.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.*, 10, 249-256.

Wilson GJ, Frantz AC, Pope LC, Roper TJ, Burke TA, Cheeseman CL, Delahay RJ (2003) Estimation of badger abundance using faecal DNA typing. *Journal of Applied Ecology*, 40, 658-666.

Chapter 3

Landscape barriers induce genetic structuring in the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps – Implications for the management of the species

G. Jacob*[1,2], K. Bollmann[1], B. Schmid[2] and F. Gugerli[1]

[1] Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

[2] Institute of Environmental Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Abstract

Habitat loss and fragmentation may affect population persistence by decreasing the size of habitat patches and increasing distances among the remaining patches. The capercaillie, *Tetrao urogallus* L., is a forest grouse species that has experienced a dramatic decline in central Europe in the last decades. In Switzerland, the capercaillie population has decreased both in numbers and in distribution range, and the species nowadays persists in geographically isolated regions. We investigated how geographical distances and topographical barriers influence the genetic relationship, based on twelve nuclear microsatellite loci, among 26 local *T. urogallus* populations distributed in five regions in the Swiss Alps. The 148 genotypes identified from non-invasive sampling were assigned to four genetic clusters. Three clusters dominated one region, whereas one was found in two regions.

We found a source–sink population constellation, in which the source region is separated from the sink regions by high mountain ranges. Topographical constraints on individual dispersal result in asymmetrical gene flow between the source and sink regions. Two of the sink regions are connected by a network of small local populations occupying geographically isolated habitat patches within a matrix of unsuitable habitat. The populations of the sink region separated from the source population by the largest geographical distances showed the lowest levels of genetic variability. The recent range contraction of the capercaillie in the source region may have further constrained the genetic connectivity with the sink regions. This might, in turn, disrupt the genetic connectivity among the remaining regions and jeopardize the persistence of *T. urogallus* in the Swiss Alps. Based on the genetic analyses of capercaillie local populations in the Swiss Alps, we recommend that high priority should be given to promote or restore the source–sink population dynamics and to maintain or promote the genetic connectivity among the sink regions. In the present study, we show that non-invasive sampling and molecular methods could be used to assess the genetic structure of populations and to identify key areas for genetic population management.

Keywords - isolation by distance; landscape barriers; population genetic structure; stepping-stone populations; *Tetrao urogallus*

Introduction

Habitat loss and fragmentation, characteristic processes in agricultural landscapes, result in decreased patch sizes and connectivity among the remaining patches (Andrén 1994). The breakdown of large-scale genetic connectivity may result in the genetic isolation of the local populations occupying these patches. Population sub-structuring and isolation generally results in the loss of genetic variability and may impact the sustainability of the local populations (Fahrig 2002; Sih et al. 2000). Local extinctions may decrease the probability of sustaining the population on a large scale, especially if new colonisation events are unlikely owing to the loss of suitable habitat. Empirical studies have shown that restoration of connectivity by translocations may promote the recovery of populations severely impacted by inbreeding depression (Franzreb 1997; Hedrick 1995; Madsen et al. 1999; Westemeier et al. 1998). Thus, monitoring the genetic variability within populations provides wildlife managers with a powerful tool to detect loss of genetic variability and to monitor the effects of

management actions (Franzreb 1997; Friar et al. 2001; Madsen et al. 1999; Westemeier et al. 1998).

Apart from habitat deterioration, natural and man-made landscape features may also affect genetic connectivity among populations by constraining individual dispersal (Gerlach and Musolf 2000; Keyghobadi et al. 1999; Lugon-Moulin and Hausser 2002). The strength of the genetic isolation by landscape barriers depends on the species' ability to disperse and on the presence of dispersal corridors (Haddad 2000). Coulon et al. (2004) calculated the least cost distance between individual roe deer, *Capreolus capreolus* L., *i.e.* the distance between individuals that minimises movements outside of secure forested areas. These authors showed that that roe deer preferably moved among habitat patches using forested corridors and, thus, that the least cost distance better mirrored the genetic relatedness among individuals than the Euclidean distance. The correlation between the genetic and the least cost distances among populations may be used to investigate the factors influencing the genetic differentiation among populations, including isolation by distance or by landscape structures.

The demography and range extension of several grouse species have been dramatically impacted by habitat change on a continental scale during the last glaciations (Drovetski and Ronquist 2003; Lucchini et al. 2001). Thus, the distribution ranges of boreal species, such as *Lagopus mutus* L., *Tetrao tetrix* L. and *T. urogallus*, have become restricted to mountainous areas in Western and Central Europe, whereas they are nowadays distributed over large and continuous forests in Scandinavia and Russia (Beaman and Madge 1998). Yet, contrasting patterns of genetic structure and levels of genetic variability have been shown between populations living in continuous *vs.* fragmented habitat in these three species (Caizergues et al. 2003a; 2003b; Segelbacher et al. 2003a). Segelbacher and co-authors (2002; 2003b) described a metapopulation structure for *T. urogallus* in the Alps and suggested a recent population decline that impacted edge more than core populations. These authors measured higher levels of genetic differentiation among populations situated in the core than those in the edge area of the metapopulation and suggested that topographical barriers had promoted the genetic differentiation among populations. Segelbacher et al. (2003b) reported a source–sink population dynamics in a peripheral area of the species' distribution in the Alps. Rutkowsky et al. (2005) showed moderate levels of genetic differentiation among four Polish *T. urogallus* local populations separated by large geographical distances. Liukkonen-Anttila et al. (2004) and Regnaut (2004) suggested that the lack of genetic differentiation among *T.*

urogallus populations in Finland and in the Pyrenean Mountains resulted from the recent range expansion of the species in these areas.

In the Swiss Alps, the capercaillie population experienced a rapid decline in numbers and distribution ranges during the last 30 years (Marti 1986; Mollet et al. 2003) and the species is nowadays restricted to large mountainous forests, geographically isolated from each other by large distances of unsuitable habitat and topographical barrier (Mollet et al. 2003).

The effect of fragmentation on the population genetic structure has been shown at the continental scale, but at a purely spatial level (Segelbacher et al. 2003a; Segelbacher and Storch 2002). Yet, little is known of the biological and topographical factors influencing dispersal and gene flow among neighbouring local populations of capercaillie at the regional scale. In this study, we investigated (i) the genetic structure of the *T. urogallus* population in the central and eastern Swiss Alps and (ii) the distribution of genetic variation among local populations. By doing this, we wished to detect barriers to gene flow and to identify regions of particular relevance for the management of the species in the Swiss Alps.

Material and methods

The study area

The size of our study area was approximately 150x100 km. Sampling was conducted in five geographically isolated regions (A-D and S, Fig. 1) situated in four core areas (3, 4a, 4b and 5, inset in Fig. 1) of the distribution range of the capercaillie as described by Mollet et al. (2003). Habitat characteristics varied largely among regions with respect to topography and vegetation cover (Bollmann et al. 2005b; Graf 2005). Mollet et al. (2003) indicated three putative landscape barriers for the capercaillie in our study area, a large and continuous area of unsuitable habitat between regions A and S, a mountain range extending between regions B and C, and a mountain range considerably higher than the natural limit of the forest between regions C and D (inset in Fig. 1). In region D, the species' distribution range extended towards Austria, where Segelbacher and Storch (2002) described the core area of the *T. urogallus* metapopulation in the Alps.

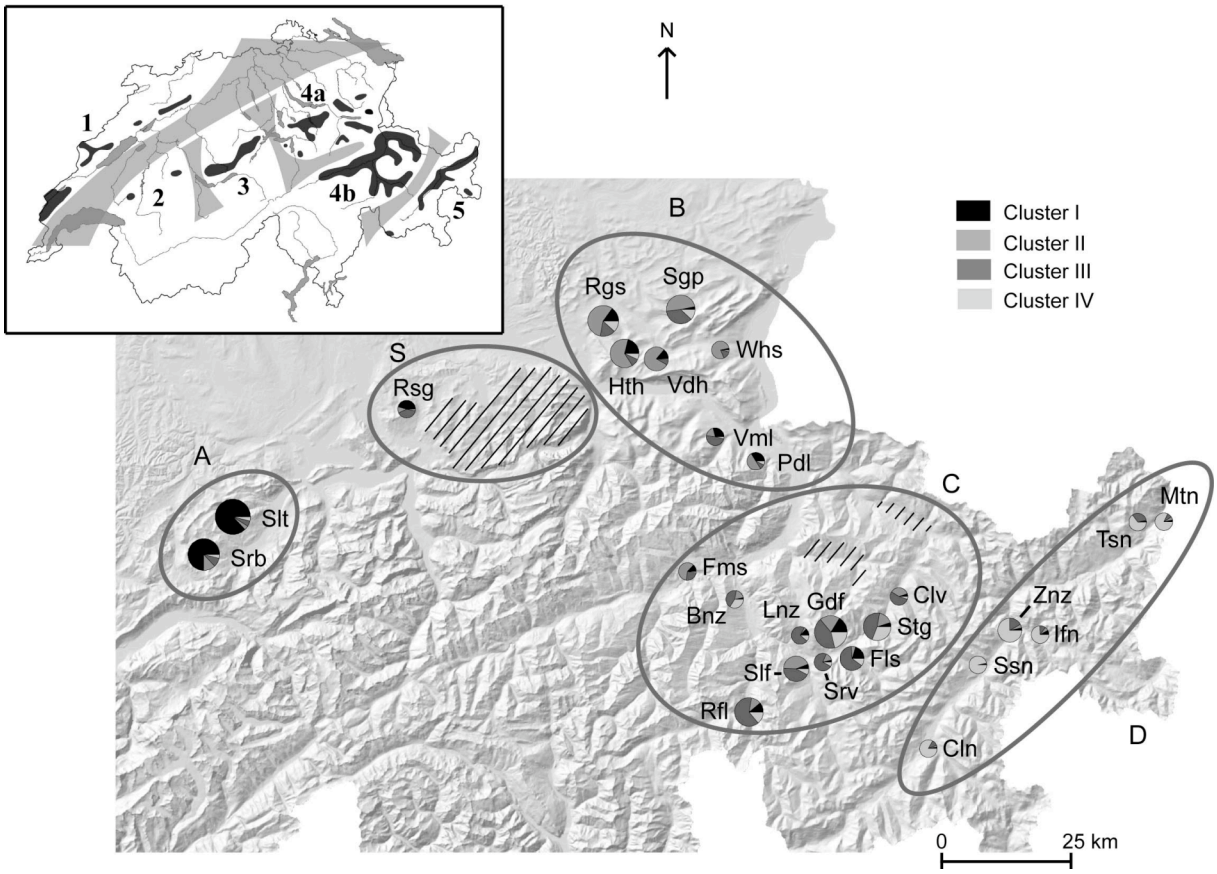

Figure 1: *Distribution of the five regions (A-D, S) and the 26 local capercaillie populations investigated in the Swiss Alps (full names and abbreviations are explained in Table 1). Hatchings in regions S and C illustrate capercaillie areas not sampled. The relative contributions of the four genetic clusters to each local population are illustrated by the pie charts (see legend). Inset: Distribution of* Tetrao urogallus *in Switzerland (dark shadings), putative barriers to gene flow (light shadings) and spatial partition into five capercaillie regions: the Jura Mountains (1), the western Prealps (2), the central Prealps (3), the eastern Prealps–central Alps (4a and 4b) and the Engadin valley and the southern valleys in the canton of Grisons (5). Map adapted from Mollet et al. (2003).*

The study species

Tetrao urogallus is a large forest grouse species with its main natural distribution range in the taiga-like forests from Scandinavia to Siberia. Outside this range, the capercaillie is restricted to mountainous areas dominated by coniferous forests in Western and Central Europe (Storch 2001).

From late winter to mid spring, capercaillie males display on lekking sites, usually situated in old-growth forest stands, on forest clearings, mires or at elevated sites. Males 4 years old and older defend the smallest territories within 1 km from the lek, whereas 1-2 year old males occupy large home ranges and do not show territorial behaviour (Storch 1997; Wegge and Larsen 1987). Females usually stay further away from the leks than males. In spring, females visit one or several leks during brief periods (Storch 1997; Wegge and Rolstad 1986), but within leks females mostly mate with the same dominant male (Storch 2001). The annual home range of females is *c*. 550 ha in the Bavarian Alps (Storch 1995), although females visiting several leks may have larger annual home ranges (Storch 1997).

Studies of capercaillie dispersal are reviewed in Storch (2001). Maximum seasonal movements of adult capercaillie were 10 km in Norway, 9 km in the German Alps and 8 km in the Pyrenees. Juvenile dispersal mostly occurs during the first autumn and the following spring. Radio-tracking studies showed that males are mostly philopatric and females tend to disperse 5-10 km, but larger distances were also observed.

Data collection

Our sampling and genotyping procedures are described in detail in Jacob et al. (submitted). The non-invasive sampling was conducted during the mating season, when we collected faecal and feather samples in the surroundings of leks (1-2 km radius). We collected 1000 samples in total, of which 530 were analysed. We divided the samples into 26 local populations (Table 1) separated by more than 2 km, that is the minimum distance among neighbouring leks reported in Central Europe, Scotland and Norway (Picozzi et al. 1992; Rolstad and Wegge 1987; Storch 1995). Two individuals were identified in fourteen of the 26 local populations ("Small") and 6–17 individuals were identified in the remaining twelve local populations ("Large"; Table 1). We screened the allelic variation at twelve nuclear microsatellite loci divided into four PCR-multiplexes. New primer pairs for ten microsatellite loci developed for the capercaillie (Segelbacher et al. 2000) were designed based on the sequences available in Genbank. We amplified two additional microsatellite loci, BG15 and BG18, developed for *T. tetrix* L. (Black grouse, Piertney and Höglund 2001). Fluorescently labelled PCR products were visualized on an ABI3100*Avant* automated sequencer (Applied Biosystems, Rotkreuz, Switzerland).

Table 1: *Full names and abbreviations (*Abb.*) of the 26 local capercaillie populations studied in the Swiss Alps, the region in which they were situated and the genetic cluster to which they were assigned: result of the assignment test from Paetkau and Strobeck (1994),* i.e. *the proportion of individuals assigned to the local population in which they were sampled,* n_{res}*, number of individuals identified as migrants,* n_{mig}*, putative local source population (indicated in brackets), distance to the putative local source population accounting for landscape barriers. See text for details.*

Populations	Abb.	Region	Inferred cluster	nres	nmig	Distance (km)
Sörenberg	Srb	A	1	12/13	1 (Slt)	9.0
Schlierental	Slt		1	17/17		
Rossberg	Rbg	S	1	2/2		
Regelstein	Rgs	B	2	10/12	1 (Fms)	65.4
					1 (Gdf)	95.7
Hinteri Höhi	Hth		2	9/10	1 (Vdh)	6.1
Vorderi Höhi	Vdh		2	5/6	1 (Whs)	12.2
Schwägalp	Sgp		2	8/10	1 (Pdl)	28.9
					1 (Bnz)	72.7
Wildhaus	Whs		2	2/2		
Vermol	Vml		3	2/2		
Pardiel	Pdl		2	2/2		
Flims	Fms	C	2	2/2		
Bonaduz	Bnz		3	2/2		
Rofla	Rfl		3	10/10		
Lenz	Lnz		3	2/2		
Got Dafora	Gdf		3	10/14	1 (Pdl)	63.8
					1 (Srb)	170.0
					1 (Stg)	8.8
					1 (Fls)	6.4
Steigwald	Stg		3	7/9	1 (Vdh)	91.1
					1 (Sgp)	101.5
Clavadel	Clv		3	2/2		
Filisur	Fls		3	6/6		
Surava	Srv		3	2/2		
Salouf	Slf		3	6/7	1 (Clv)	25.5
Celerina	Cln	D	4	2/2		
Susauna	Ssn		4	2/2		
Zernez	Znz		4	8/9	1 (Ssn)	8.9
Il Fuorn	Ifn		4	2/2		
Tschlin	Tsn		4	2/2		
Martina	Mtn		4	2/2		

Data analysis

We tested for the departure from Hardy-Weinberg equilibrium (HWE) for all loci over all local populations and within each local population using the algorithm of Guo & Thompson (1992) as implemented in Genepop 1.3 (Raymond and Rousset 1995b). The associated P-values were computed by using the default parameters for the number of batches (n=100), the dememorization steps (n=10'000) and the number of iterations (n=5'000). We did not test for linkage disequilibrium among loci, given that false positive signals were likely to result from the small sample sizes considered in the tests (Teare et al. 2002).

We used the program Structure (Pritchard et al. 2000) to investigate the genetic structure of the Swiss Alpine capercaillie population. This algorithm makes use of the information contained by individual's multi-locus genotypes to divide the data into K genetic clusters. A posterior probability of the clustering into K groups is calculated. Running the simulation for a range of K allows the determination of the value of K that best captures the genetic variance among the multi-locus genotypes. Individuals are assigned probabilistically to one or more clusters in the case of admixed individuals.

We conducted all the simulations using the admixture and correlated allelic frequencies models. We set the length of the burnin period to 100'000 iterations and collected data for the next 1'000'000 iterations. A preliminary study showed that these settings were sufficient for the estimates of α, the degree of admixture, and F, the divergence distance among populations $D_{i,j}$, to converge. For K=1–6, we ran three independent replicates to verify that the parameter estimates (α, F and Pr($X|K$), the posterior probability of the clustering into K groups) were consistent across runs. We averaged Pr($X|K$) across the three runs to calculate the posterior probability of K (Pritchard and Wen 2002). Individuals were assigned to the cluster contributing most of their inferred ancestries, taken from the run showing the highest Pr($X|K$). Local populations were assigned to the cluster contributing most of their gene pool, calculated as the average inferred ancestries of each cluster over all individuals in the local populations.

We used an analysis of molecular variance (AMOVA, Excoffier et al. 1992) framework to test for significant genetic differentiation (F_{ST}) among regions and among local populations within regions. The significance of the genetic differentiation among local populations was assessed with an exact test of population differentiation (Raymond and Rousset 1995a). Randomization was achieved by 10'000 dememorization steps followed by 100'000 steps of Markov chain. We conducted assignment tests in each local population,

based on the method of Paetkau and Strobeck (1994), to identify putative migrants and their local population of origin. Analyses were performed with Arlequin v2.001 (Schneider et al. 2000).

We used Fstat (Goudet 2001) to calculate the mean number of alleles per locus, A_o and the allelic richness (r(g), Petit et al. 1998) within local populations and regions, where g is the number of gene copies considered in the rarefaction estimate of allelic richness. Given the low sample size (n=2) in some local populations, we may have had limited power to detect changes in allelic richness among populations (Leberg 2002). We therefore calculated this parameter only in those local populations where at least six individuals were identified. We measured the proportion of heterozygous individuals, H_o, and the expected heterozygosity, H_e with GenAlEx 6 (Peakall and Smouse in press). These parameters were used to investigate the effects of the sample sizes of the local populations on the levels of genetic variation using one-way analysis of variance (ANOVA). We used locus-by-locus comparisons (Wilcoxon two sample paired-tests) to assess differences in the proportion of heterozygous individuals, expected heterozygosity and allelic richness among regions. Region S consists of several large and small patches, of which a single local population, Rossberg (n=2), was sampled (Fig. 1). We therefore excluded region S from the analyses. ANOVAs and Wilcoxon tests were performed using the R statistical package (R Development Core Team 2005).

We investigated the pairwise relationship between the regions B and C, connected by forested areas hosting small local populations of *T. urogallus*, and between the regions C and D, separated by mountain ridges several hundred meters higher than the natural limit of the forest. For the genetic differentiation among local populations, we used F_{ST} based on allele frequencies (Wright 1951), calculated with GenAlEx 6 (Peakall and Smouse in press). We used F_{ST} from allelic frequencies in this analysis, because this parameter directly relates to changes in allelic frequencies owing to the genetic contribution of dispersers. As the least cost distance among local populations, we inferred the most probable route used by *T. urogallus* to disperse between local populations. We applied the following rules: (i) *T. urogallus* disperses between occupied habitat patches along the valley slopes rather than across valleys, (ii) *T. urogallus* does not cross high mountain ranges above the natural tree line or, if so, crosses the mountain ranges at the pass lowest in altitude and closest to the local population. We calculated the coordinates of the local populations as the centroid of individual coordinates. We tested the correlation between effective geographic distance and genetic distances among

populations using Mantel tests (Mantel 1967) based on 1000 randomisations, conducted with the R statistical package (R Development Core Team 2005).

Results

Pattern of genetic diversity

Out of 530 DNA extracts from non-invasive samples analysed, 351 (66%) amplified at one or more loci, of which 244 (46%) DNA extracts successfully amplified at eight to twelve loci and were assigned to *T. urogallus*. These 244 multilocus genotypes grouped into 148 unique allelic combinations differentiated by two alleles or more at those loci that were amplified (Jacob et al. submitted).

Since null alleles might bias the estimate of *K* using Structure (Pritchard and Wen 2002), we removed three loci that deviated from HWE over all populations (TuD4, TuD6 and TuT1) and showed evidences for null alleles. Based on the nine remaining microsatellite loci, a total of 63 different alleles were detected, and the number of alleles by locus ranged from 4 to 13 (mean=7). Within local populations, the mean number of alleles per locus ranged from 1.8 in Rossberg to 4.4 in Got Dafora, and expected heterozygosity ranged from 0.319 in Rossberg to 0.648 in Got Dafora. Private alleles were found in five small and four large local populations.

We found a significant deviation from HWE (X^2=246.2, p=0.02) over all loci and local populations. One local population, Schlierental, significantly deviated from HWE (X^2=32.7, p=0.008) and showed a deficit in heterozygous individuals at locus TuT4.

Inference of the genetic structure

The classification of the Swiss Alpine capercaillie population into *K*=4 clusters (I-IV) was supported by the highest posterior probability (Pr(*X*|*K*)). All individuals and, consequently, all local populations comprised elements from clusters I-IV (Fig. 1). We sampled 30 individuals in region A, of which 28 were assigned to cluster I, and one each was assigned to clusters II and III (Fig. 1). The two individuals sampled in Rossberg (region S) were assigned to clusters I and III, respectively. In region B, 31 individuals were assigned to cluster II, six to cluster I, six to cluster III and one to cluster IV. Vermol was the only local population from region B assigned to cluster III. Two individuals were sampled in Vermol and one each was assigned to cluster II and cluster III. From the 56 individuals sampled in

region C, 36 were assigned to cluster III, four to cluster I, nine to cluster II and seven to cluster IV. Flims was the only local populations in region C assigned to cluster II. In Flims, one individual each were assigned to cluster II and cluster III. In region D, almost all individuals were assigned to cluster IV, but two were assigned to cluster III.

The analysis of the inferred genetic structure revealed that 6.6% (AMOVA, $p<0.001$) of the total genetic variation was explained by the genetic differentiation among regions, and 4.9% (AMOVA, $p<0.001$) resulted from the genetic differentiation among local populations within regions. Pairwise differentiations between regions A and B (F_{ST}=0.074), regions B and C (F_{ST}=0.031) and regions C and D (F_{ST}=0.063) were significant (AMOVA, p=0.01), as all other pairwise comparisons between regions (data not shown). We also observed significant F_{ST} values between some local populations situated in different regions (data not shown). In contrast, we found no significant differences in allelic frequencies between regions or between local populations (exact test of population differentiation, Raymond and Rousset 1995a).

Based on the assignment test of Paetkau and Strobeck (1994), eight individuals (5% of n=148; Table 1) were assigned to local populations other than those in which they were found, but situated in the same region. The distance between the local populations in which they were sampled and those to which they were assigned was 13.2 ± 8.8 km (mean + SE) and up to 28.9 km. Six individuals (4% of n=148) were sampled in a local population situated in the region B (or C) and assigned to a local population situated in the region C (or B, respectively). One capercaillie sampled in Got Dafora (region C) was assigned to Sörenberg (region A), at 170 km distance.

Within local populations, increasing sample size had a positive impact on the mean number of alleles detected per locus (A_o; ANOVA, $p<0.001$) and on the expected heterozygosity (H_e; ANOVA, p=0.02), but had no significant effect on the proportion of heterozygous individuals, H_o, and on the allelic richness calculated for large local populations, $r(8)$ (Table 2). Allelic richness calculated within regions, $r(28)$, decreased with increasing geographic and genetic isolation of the regions and was significantly lower in region A than in region B (Wilcoxon, p=0.04) and region C (Wilcoxon, p=0.03). Expected heterozygosity was lower in region A than in region C (Wilcoxon, p=0.008). The proportion of heterozygous individuals in region A was significantly lower than in region B (Wilcoxon, p=0.02), in region C (Wilcoxon, p=0.004) and in region D (Wilcoxon, p=0.012).

Table 2: *List of the 26 local capercaillie populations studied in the Swiss Alps and their genetic characteristics: sample size,* n*, mean number of alleles per locus,* A_o*, proportion of heterozygous individuals,* H_o*, expected heterozygosity,* H_e*, allelic richness calculated for four diploid individual,* r*(8). The size class of the local populations, "Large" (*n ≥ *6) or "Small" (*n = *2) and region (cf. Table 1) were used as fixed factors in the analysis of variance (ANOVA; see text).*

Populations	*n*	A_o	H_o	H_e	*r*(8)	Size class
Sörenberg	13	3.8	0.462	0.491	2.73	Large
Schlierental	17	3.6	0.485	0.503	2.67	Large
Rossberg	2	1.8	0.500	0.319	-	Small
Regelstein	12	3.9	0.648	0.595	3.08	Large
Hinteri Höhi	10	3.8	0.689	0.586	3.08	Large
Vorderi Höhi	6	3.2	0.607	0.504	2.85	Large
Schwägalp	10	3.7	0.635	0.584	3.10	Large
Wildhaus	2	2.8	0.722	0.528	-	Small
Vermol	2	2.4	0.722	0.500	-	Small
Pardiel	2	2.4	0.611	0.486	-	Small
Flims	2	2.4	0.500	0.514	-	Small
Bonaduz	2	2.4	0.556	0.458	-	Small
Rofla	10	3.3	0.607	0.490	2.65	Large
Lenz	2	2.2	0.500	0.431	-	Small
Got Dafora	14	4.4	0.661	0.648	3.40	Large
Steigwald	9	3.3	0.585	0.564	2.88	Large
Clavadel	2	2.6	0.722	0.542	-	Small
Filisur	6	3.6	0.722	0.596	3.24	Large
Surava	2	3.0	0.889	0.597	-	Small
Salouf	7	3.2	0.717	0.590	2.98	Large
Celerina	2	2.2	0.722	0.458	-	Small
Susauna	2	2.3	0.722	0.514	-	Small
Zernez	6	3.9	0.652	0.587	3.36	Large
Il Fuorn	2	2.3	0.833	0.500	-	Small
Tschlin	2	2.0	0.278	0.389	-	Small
Martina	2	2.0	0.667	0.389	-	Small
Region A	30	4.2	0.472	0.508	3.74[a]	
Region B	44	5.2	0.652	0.631	4.52[a]	
Region C	56	5.6	0.646	0.659	4.50[a]	
Region D	16	4.8	0.642	0.630	4.70[a]	

[a] calculated for 14 diploid individuals, *r*(28)

Relationship between geographic and genetic distance

We detected no influence of the geographic distances on the genetic differentiation between regions B and C (Mantel test, p=0.659; Fig 2a and 2b). This pattern is congruent with

the low differentiation (F_{ST}=0.031) calculated between regions B and C. We observed a significant relationship between geographic and genetic distances between regions C and D (Mantel test, p<0.001; Fig. 2c and 2d). Small local populations showed on average higher levels of genetic differentiation than the large local populations in regions B and C (ANOVA, p<0.001; Fig. 3a) and in regions C and D (ANOVA, p<0.001; Fig. 3b).

Discussion

Genetic structure of the capercaillie population in the Swiss Alps

Mollet et al. (2003) suggested that the *Tetrao urogallus* population in Switzerland is divided into five regional entities. In the present study, we found that local populations of *T. urogallus* situated in the distribution areas 3, 4a, 4b and 5 were genetically structured into four genetic clusters. We conducted non-invasive sampling in five regions, each consisting of a mountain range or a valley where we knew, from field evidence, that habitat quality and individual density were high. The genetic clusters I, II, III and IV dominated the regions A, B, C and D, respectively.

Segelbacher and Storch (2002) inferred that *T. urogallus* populations in the Alps are organised as a metapopulation. The authors suggested that topographical barriers constrained individual dispersal among core populations and that the recent population decline was accompanied by an increased isolation among edge populations. Based on field surveys and genetic data, Segelbacher et al. (2003b) described a source–sink dynamic among populations situated in peripheral areas of the species distribution. The Engadin valley (capercaillie region 5, inset Fig. 1) is topographically connected to the central Austrian Alps, *i.e.* the core area of the capercaillie metapopulation described by Segelbacher and Storch (2002). In the present study, we show that region D had the greatest level of allelic richness, although the difference with the neighbouring region C was not significant. This result suggests that region D is not only geographically, but also genetically connected to the core area in the Austrian Alps and may act as a source population for region C. Local populations in region C comprised a larger contribution from cluster IV than did local populations in region D from cluster III. Ferreras (2001) suggested that asymmetrical connectivity among *Lynx pardinus* L. (Iberian lynx) populations may result from the configuration of habitats encountered by dispersers, which forces dispersers from the source populations to cross unsuitable habitat patches. A habitat model of Graf et al. (2004; 2005) shows the presence of suitable habitat patches along the

slopes of the Albula valley (region C), which is connected to region D via a pass, Pass d'Alvra/Albulapass (2312 m). We therefore suggest that region D is a source population for region C and that source–sink dispersal through the Pass d'Alvra/Albulapass resulted in asymmetrical gene flow between regions C and D.

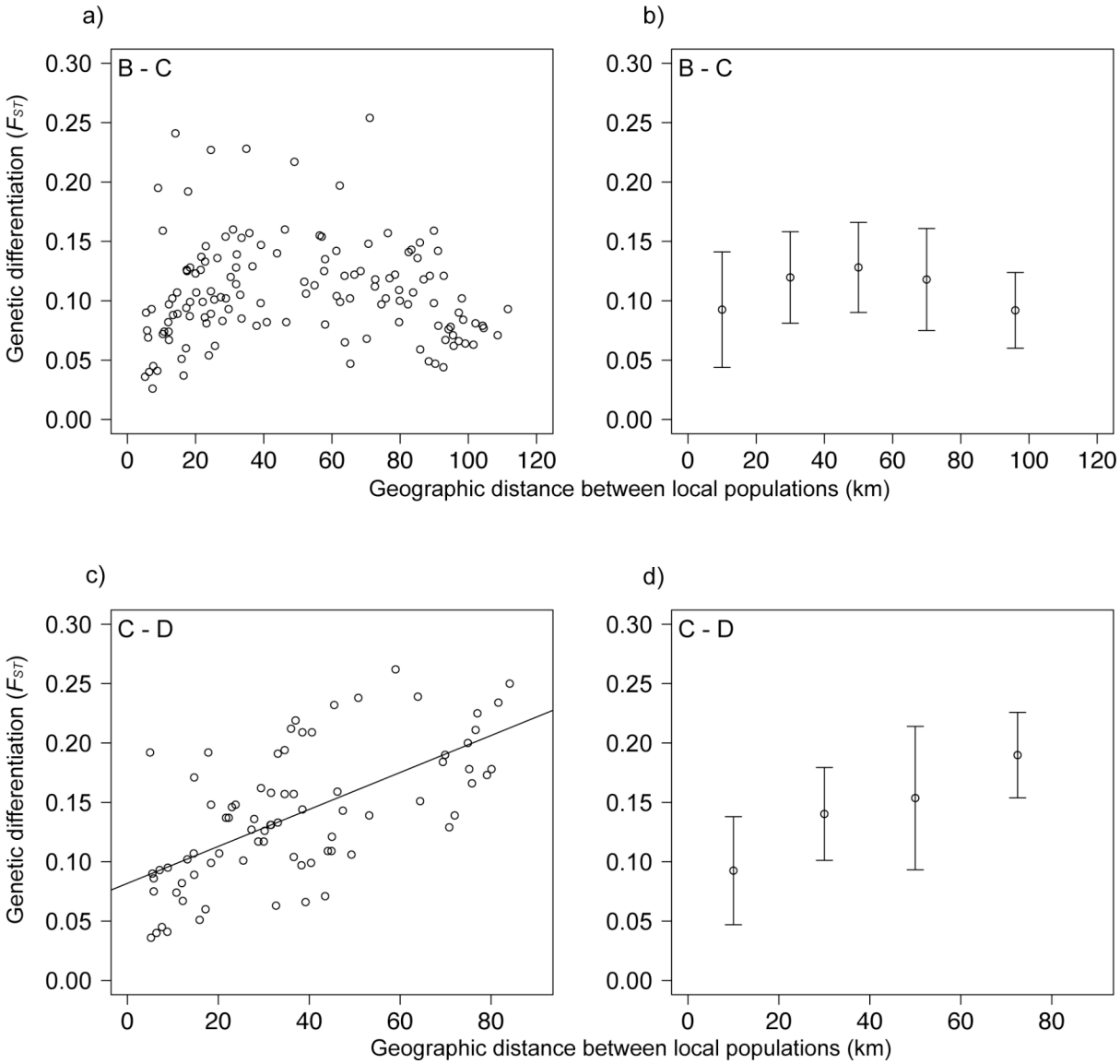

Figure 2: *Scatter plot of the genetic differentiation* (F_{ST}) *against the geographic distance between local capercaillie populations (*a *and* c*) and mean genetic differentiation across 20-km distance classes (*b *and* d*).* a *and* b*: comparison between local populations from regions B and C;* c *and* d*: comparison between local populations from regions C and D.*

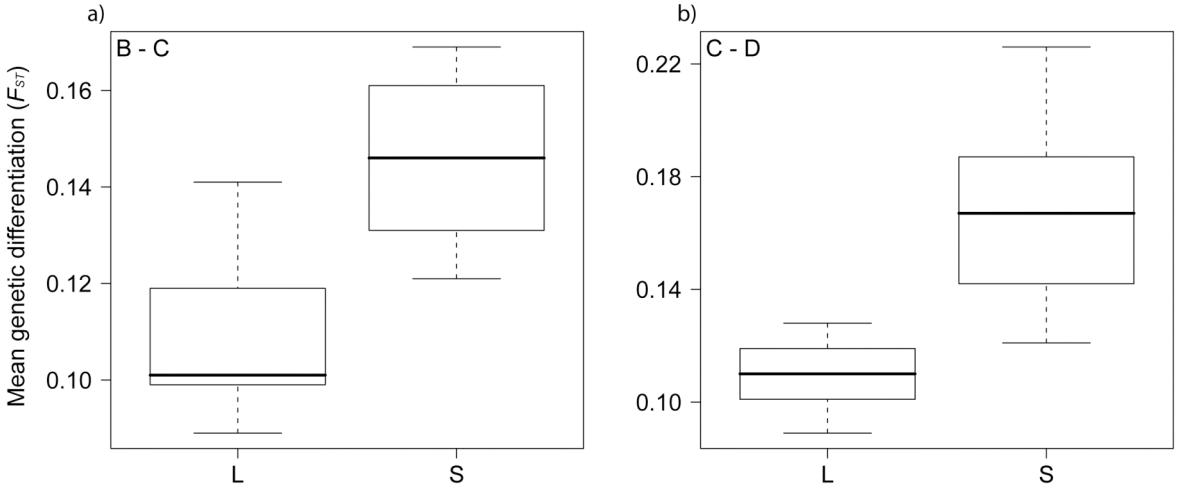

Figure 3: *Box-whisker plot of the mean genetic differentiation, measured as* F_{ST}*, in the large (L) and small (S) population size classes of capercaillie in regions B and C (*a*) and in regions C and D (*b*).*

Empirical studies have demonstrated the role of natural and artificial corridors in inter-population dispersal (Haas 1995; Haddad 2000). We observed significant F_{ST} (AMOVA) values between regions B and C, but no difference in the level of allelic richness. Cluster III contributed approximately the same proportion of the gene pool in region B as cluster II did in region C. This suggests that the two regions were genetically admixed and that gene flow was likely to be symmetrical between these two regions. Surprisingly, we observed the highest values in the genetic differentiation, measured as F_{ST} from allelic frequencies, at intermediate distance classes (Fig. 2a). Small local populations had a significantly higher average level of genetic differentiation than large ones. The contribution of the alleles carried by a migrant to the allele frequencies of the local population where it establishes (=1/n) is inversely proportional to the population size, ranging from 100% in the extreme case of a founder event to 0% if the population has an infinite size. Thus, migration events into small local populations may have increased the variance in allelic frequencies among local populations and the levels of genetic differentiation, F_{ST} calculated from allelic frequencies, among those. We therefore suggest that F_{ST} values increased at intermediate distance classes as a result of dispersal events among small local populations rather than genetic isolation among those. Thus, regions B and C may be regarded as semi-isolated regions connected by a

network of small local populations. The assignment test of Paetkau and Strobeck (1994) suggested that some migrants originated from small local populations and may provide further evidence that Pardiel, Vermol, Flims and Bonaduz may be stepping-stone populations connecting regions B and C. Moreover, we observed an abandoned nest in Vermol, which proved that reproduction and juvenile dispersal from these local populations may occur. However, the assumption of equal local populations sizes for the assignment test was violated in our study (n=2–17) and may have resulted in a bias. Small local populations were characterised by a large variance in allelic frequencies and, therefore, the risk exists that small local populations were designated as source populations by chance. We have, at present, no evidence to favour one of these two scenarios. Paternity analyses or coalescent analyses may provide additional information on the direction of gene flow and on the source populations. However, the nine markers used in this study did not show sufficient variability to conduct these analyses. The assignment procedure provided by the program Structure performed well in identifying the admixture zone between regions B and C. However, because of the weak genetic differentiation among local populations, this assignment test had a low spatial resolution.

Region S consisted of several large and small patches of which a single local population, Rossberg (n=2), was sampled (Fig. 1). Owing to its geographical position and extent toward the east, region S might be genetically differentiated from the neighbouring regions A and B. Therefore, the present study should be viewed as a conservative estimate of the level of genetic structure of the *T. urogallus* population in the Swiss Alps. Additional sampling is required in region S to study its genetic characteristics and to infer the genetic relationship among regions A, S and B.

In the present study, we found that low sample sizes may result in a large variance in expected heterozygosity. Although this parameter should be robust to compare populations differing in sample size (Nei 1973), our results suggest that it might not be suitable if populations vary greatly in sample sizes. Similarly, allelic richness calculated for one diploid individual, $r(2)$, *i.e.* including the smallest local population, was negatively correlated with increasing sample size (data not shown). The influence of sample size was not yet detectable when allelic richness was calculated for four diploid individuals, $r(8)$. These results suggest that a minimum sample size is required and that small populations, n=2 in the present study, should be excluded to limit the bias in the estimates of allelic richness (Leberg 2002).

Implications for the conservation and management of the species

Since the 1950s the capercaillie experience a dramatic decline in Switzerland. Marti (1986) and Mollet et al. (2003) quantified this trend for the last 30 years. The species disappeared from the lowland areas of its distribution range, leading to the breakdown of the former continuous population into geographically isolated capercaillie regions (Mollet et al. 2003). In the present study, we suggest that region D is connected to the *T. urogallus* metapopulation in Austria described by Segelbacher and Storch (2002). Mollet et al. (2003) reported the recent range contraction and population decline of the capercaillie in the Upper Engadin valley, *i.e.* in the area of the most probable dispersal corridor between regions C and D. Our results suggest that regions C and D have been historically connected. However, we found no evidence of a recent dispersal event between regions C and D. The apparent discrepancy between the past and present levels of gene flow, estimated from different statistical methods, suggests that the recent decline of the capercaillie in region D further constrained the genetic connectivity between regions C and D. Based on a habitat model and a validation procedure by presence–absence data, Graf (2005) showed that habitat degradation impacted the persistence of capercaillie local populations in the Swiss Alps. As shown in general and specifically for the capercaillie (Bollmann et al. 2005a; Fahrig and Merriam 1985), local populations occupying small habitat patches have a greater probability of extinction owing to small population sizes and reduced connectivity. Thus, the range contraction in regions B or C might have a negative impact on the number of emigrants dispersing from these regions and decrease the probability of persistence of the network of small and geographically isolated local populations connecting these regions. Genetically isolated populations experience higher rates of fixation of deleterious alleles and of loss of alleles by genetic drift (Frankham 1995). Region A was isolated early following the capercaillie distribution range contraction (Mollet et al., 2003) and showed significantly lower levels of allelic richness and proportion of heterozygous individuals than did regions B, C and D. Genetic isolation of regions B and C may result in a similar decrease of the genetic variability. Isolated populations may be severely impacted by inbreeding depression (Madsen et al. 1996; Saccheri et al. 1998; Spielman et al. 2004; Westemeier et al. 1998). However, empirical studies have shown that the restoration of genetic connectivity by translocations of individuals may promote the recovery/demographic expansion of populations severely impacted by inbreeding depression (Franzreb 1997; Hedrick 1995; Madsen et al. 1999; Spielman and Frankham 1992; Westemeier et al. 1998).

Based on the genetic analyses of local capercaillie populations in the Swiss Alps, we recommend that management actions for *T. urogallus* in the Swiss Alps focus on restoring and promoting connectivity between regions. High priority should be given to maintain the source–sink population dynamics between regions C and D and to improve and restore habitats and local populations along the corridor connecting regions B and C. General measures that improve habitat quality in regions A and B will further maintain connectivity among habitat patches within and among regions A and B. Additional sampling is required to infer the population dynamics among regions A, S and B and to assess the management actions to be planned in these regions.

Our results suggest that the persistence of peripheral *T. urogallus* populations, including the study population in the Swiss Alps, may be jeopardized by the decline of the metapopulation in the eastern Alps reported by Segelbacher and Storch (2002). We therefore recommend that international collaborative management actions are undertaken over the entire species distribution range to maintain or increase habitat patch size and quality within regions, and to restore and promote connectivity among regions in the Alps.

References

Andrén, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos 71:355-366.

Beaman, M., and S. Madge. 1998. The handbook of bird identification for Europe and the western paleartic, Princeton, New jersey.

Bollmann, K., R. F. Graf, and W. Suter. 2005a. Assessing the role of patch area, isolation, and connectivity for predicting capercaillie occurrence in the Alps. XXVIIth Congress of the International Union of Game Biologists, Hanover. Book of extended abstracts:61-62.

Bollmann, K., P. Weibel, and R. F. Graf. 2005b. An analysis of central Alpine capercaillie spring habitat at the forest stand scale. Forest Ecology and Management 215:307-318.

Caizergues, A., A. Bernard-Laurent, J.-F. Brenot, L. Ellison, and J. Y. Rasplus. 2003a. Population genetic structure of rock ptarmigan *Lagopus mutus* in Northern and Western Europe. Molecular Ecology 12:2267-2274.

Caizergues, A., O. Ratti, P. Helle, L. Rotelli, L. Ellison, and J. Y. Rasplus. 2003b. Population genetic structure of male black grouse (*Tetrao tetrix*) in fragmented vs. continuous landscape. Molecular Ecology 12:2297-2305.

Coulon, A., J. F. Cosson, J. M. Angibault, B. Cagnelutti, M. Galan, N. Morellet, E. Petit, S. Aulagnier, and A. J. M. Hewison. 2004. Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Molecular Ecology 13:2841-2850.

Drovetski, S. V., and F. Ronquist. 2003. Plio-Pleistocene climatic oscillations, holarctic biogeography and speciation in an avian subfamily. Journal of Biogeography 30:1173-1181.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fahrig, L. 2002. Effect of habitat fragmentation on the extinction threshold: A synthesis. Ecological Applications 12:346-353.

Fahrig, L., and G. Merriam. 1985. Habitat patch connectivity and population survival. Ecology 66:1762-1768.

Ferreras, P. 2001. Landscape structure and asymmetrical inter-patch connectivity in a metapopulation of the endangered Iberian lynx. Biological Conservation 100:125-136.

Frankham, R. 1995. Conservation genetics. Annual Revue of Genetics 29:305-327.

Franzreb, K. E. 1997. Success of intensive management of a critically imperiled population of Red-cockaded Woodpeckers in South Carolina. Journal Of Field Ornithology 68:458-470.

Friar, E. A., D. L. Boose, T. LaDoux, E. H. Roalson, and R. H. Robichaux. 2001. Population structure in the endangered Mauna Loa silversword, *Argyroxiphium kauense* (Asteraceae), and its bearing on reintroduction. Molecular Ecology 10:1657-1663.

Gerlach, G., and K. Musolf. 2000. Fragmentation of landscape as a cause for genetic subdivision in bank voles. Conservation Biology 14:1066-1074.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, Lausanne, Switzerland.

Graf, R. F. 2005. Analysis of capercaillie habitat at the landscape scale using aerial photographs and GIS. Pp. 143. Swiss Federal Institute of Technology, Zurich, Zurich.

Graf, R. F., K. Bollmann, W. Suter, and H. Bugmann. 2004. Using a multi-scale model for identifying priority areas in capercaillie (*Tetrao urogallus*) conservation. Pp. 84-90 *in* R. Smithers, ed. Proceedings of the 12th annual IALE(UK) conference "Landscape ecology of trees and forest", Cirencester, UK.

Graf, R. F., K. Bollmann, W. Suter, and H. Bugmann. 2005. The importance of spatial scale in habitats model: capercaillie in the Swiss Alps. Landscape Ecology 20:703-717.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

Haas, C. A. 1995. Dispersal and use of corridors by birds in wooded patches on an agricultural landscape. Conservation Biology 9:845-854.

Haddad, N. 2000. Corridor length and patch colonization by a butterfly, *Junonia coenia*. Conservation Biology 14:738-745.

Hedrick, P. W. 1995. Gene flow and genetic restoration - the Florida Panther as a case-study. Conservation Biology 9:996-1007.

Jacob, G., R. Debrunner, F. Gugerli, B. Schmid, and K. Bollmann. submitted. Estimating the population census sizes of capercaillie (*Tetrao urogallus* L., Aves) in the Swiss Alps from single-occasion genetic capture-recapture data.

Keyghobadi, N., J. Roland, and C. Strobeck. 1999. Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). Molecular Ecology 8:1481-1495.

Leberg, P. L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. Molecular Ecology 11:2445-2449.

Liukkonen-Anttila, T., O. Ratti, L. Kvist, P. Helle, and M. Orell. 2004. Lack of genetic structuring and subspecies differentiation in the capercaillie (*Tetrao urogallus*) in Finland. Annales Zoologici Fennici 41:619-633.

Lucchini, V., J. Höglund, S. Klaus, J. Swenson, and E. Randi. 2001. Historical biogeography and a mitochondrial DNA phylogeny of grouse and ptarmigan. Molecular Phylogenetics and Evolution 20:149-162.

Lugon-Moulin, N., and J. Hausser. 2002. Phylogeographical structure, postglacial recolonization and barriers to gene flow in the distinctive Valais chromosome race of the common shrew (*Sorex araneus*). Molecular Ecology 11:785-794.

Madsen, T., R. Shine, M. Olsson, and H. Wittzell. 1999. Conservation biology - restoration of an inbred adder population. Nature 402:34-35.

Madsen, T., B. Stille, and R. Shine. 1996. Inbreeding depression in an isolated population of adder *Vipera berus*. Biological Conservation 75:113-118.

Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. Cancer Research 27:209-220.

Marti, C. 1986. Verbreitung und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz. Der Ornithologische Beobachter 83:67-70.

Mollet, P., B. Badilatti, K. Bollmann, R. F. Graf, R. Hess, H. Jenny, B. Mulhauser, A. Perrenoud, F. Rudmann, S. Sachot, and J. Studer. 2003. Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. Der Ornithologische Beobachter 100:67-86.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Science of the United States of America 70:3321-3323.

Paetkau, D., and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3:489-495.

Peakall, R. O. D., and P. E. Smouse. in press. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes

Petit, R. J., A. El Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12:844-855.

Picozzi, N., D. C. Catt, and R. Moss. 1992. Evaluation of capercaillie habitat. Journal of Applied Ecology 29:751-762.

Piertney, S. B., and J. Höglund. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Notes 1:303-304.

Pritchard, J. K., M. Stephens, and P. Donnely. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Pritchard, J. K., and W. Wen. 2002. Documentation for *structure* software: Version 2.

R Development Core Team. 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raymond, M., and F. Rousset. 1995a. An exact test for population differentiation. Evolution 49:1280-1283.

Raymond, M., and F. Rousset. 1995b. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Regnaut, S. 2004. Population genetics of Capercaillie (*Tetrao urogallus*) in the Jura and the Pyrenees: a non-invasive approach to avian conservation genetics. Pp. 180. Faculté de Biologie et de Médecine. University of Lausanne, Lausanne.

Rolstad, J., and P. Wegge. 1987. Distribution and size of capercaillie leks in relation to old forest fragmentation. Oecologia 72:389-394.

Rutkowsky, R., H. Niewęgłowski, R. Dziedzic, M. Kmieć, and J. Goździewski. 2005. Genetic variability of Polish population of the Capercaillie *Tetrao urogallus*. Acta Ornithologica 40:27-34.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491-494.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: A software for population genetic data analysis., Genetics and Biometry laboratory, University of Geneva, Switzerland.

Segelbacher, G., J. Höglund, and I. Storch. 2003a. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology 12:1773-1780.

Segelbacher, G., R. J. Paxton, G. Steinbruck, P. Trontelj, and I. Storch. 2000. Characterization of microsatellites in capercaillie *Tetrao urogallus*. Molecular Ecology 9:1934-1935.

Segelbacher, G., and I. Storch. 2002. Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. Molecular Ecology 11:1669-1677.

Segelbacher, G., I. Storch, and J. Tomiuk. 2003b. Genetic evidence of capercaillie *Tetrao urogallus* dispersal sources and sinks in the Alps. Wildlife Biology 9:267-273.

Sih, A., B. G. Jonsson, and G. Luikart. 2000. Habitat loss: ecological, evolutionary and genetic consequences. Trends in Ecology & Evolution 15:132-134.

Spielman, D., B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America 101:15261-15264.

Spielman, D., and R. Frankham. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations - Improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. Zoo Biology 11:343-351.

Storch, I. 1995. Annual home ranges and spacing patterns of Capercaillie in Central Europe. Journal of Wildlife Management 59:392-400.

Storch, I. 1997. Male territoriality, female range use, and spatial organisation of capercaillie *Tetrao urogallus* leks. Wildlife Biology 3:149-161.

Storch, I. 2001. *Tetrao urogallus* Capercaillie. BWP Update 3:1-24.

Teare, M. D., A. M. Dunning, F. Durocher, G. Rennart, and D. F. Easton. 2002. Sampling distribution of summary linkage disequilibrium measures. Annals Of Human Genetics 66:223-233.

Wegge, P., and B. B. Larsen. 1987. Spacing of adult and subadult male Common capercaillie during the breeding-season. Auk 104:481-490.

Wegge, P., and J. Rolstad. 1986. Size and spacing of capercaillie leks in relation to social-behavior and habitat. Behavioral Ecology And Sociobiology 19:401-408.

Westemeier, R. L., J. D. Brawn, S. A. Simpson, T. L. Esker, R. W. Jansen, J. W. Walk, E. L. Kershner, J. L. Bouzat, and K. N. Paige. 1998. Tracking the long-term decline and recovery of an isolated population. Science 282:1695-1698.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323-354.

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Chapter 4

Genetic signature of the recent decline and fragmentation of the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps

G. Jacob*[1,2] and B. Schmid[2]

[1] Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

[2] Institute of Environmental Sciences, University of Zurich, Switzerland

Abstract

Studying the genetic composition of neighbouring populations allows the inference of the genetic connectivity between them and, thus, may be of value to elaborate management plans for endangered species. The capercaillie (*Tetrao urogallus* L.) is a forest grouse species that experienced a dramatic decline in Switzerland during the last century as a result of changes in forestry practices and increasing human-induced disturbances. To study the dynamics of the capercaillie genetic structure in the Swiss Alps, we analysed *T. urogallus* specimens collected before the species' pronounced and continuous decline since the 1950s, and between 2001–2004. We found significant differeneces between past and present population genetic structure within regions but smaller differences between regions. This suggests that *T. urogallus* consisted of a network of genetically connected regions in the Swiss Alps. Since the 1950s, the genetic connectivity between regions has declined. We also

found evidence that genetic drift following the species' demographic bottleneck induced a shift in allelic frequencies between the two time periods studied. Observed heterozygosity (H_o) of individuals was significantly higher in the past than in the in present, but expected heterozygosity (H_e) and allelic richness have not yet declined. These results suggest that it is still time to restore genetic diversity and connectivity in the capercaillie in the Swiss Alps by maintaining or improving the quality of the remaining habitat patches and increasing gene flow via appropriate habitat management between habitat patches.

Keywords – ancient DNA, genetic connectivity, heterozygosity, nuclear microsatellite

Introduction

Two main genetic causes are proposed to explain the loss of individuals fitness in declining populations: the fixation of deleterious alleles (Lande 1994) and the loss of allelic diversity that constrain the sustainability of population in a changing environment (Frankham 2005). Empirical evidence supports the notion that genetic impoverishment plays an eminent role in the process of population decline and extinction (Madsen et al. 1996; Westemeier et al. 1998; Hansson & Westerberg 2002; Keller & Waller 2002; Spielman et al. 2004). Thus, monitoring the dynamics of the genetic variability within populations over time offers the opportunity to detect decrease in genetic variability within and in connectivity among populations and, thus, is of value to elaborate management plans for endangered species (Hedrick 1995; Madsen et al. 1999; 2004).

Assessing the level of genetic diversity in past populations from museum specimens made it possible to monitor changes in population genetic variability following bottleneck events or population declines (Ellegren 1994; Bouzat et al. 1998a; Bouzat et al. 1998b; Groombridge et al. 2000; Gautschi 2001) and to estimate the long-term effective population size of populations (Miller & Kapuscinski 1997; Johnson et al. 2003; Miller & Waits 2003). Comparing the genetic composition within populations over time may also provide information on the breeding biology of the study species. In their study of the Atlantic salmon, *Salmo salar* L., Nielsen (1997) showed that past and present specimens collected in the same river streams were more closely related to each other than to specimens collected in other river streams. This result suggested a high site-fidelity and limited gene flow among local populations. In contrast, Johnson et al. (2004) showed that local populations of the Greater prairie-chicken, *Tympanuchus cupido* L., tended to cluster by period of sampling

(past *vs*. present) rather than by geographical location, which suggested that past local populations were connected. These authors reported the split of the present population into two genetically differentiated units, owing to the recent fragmentation of the habitat of the species and its general decline in numbers.

Habitat loss and fragmentation decrease the landscape connectivity among regions (Andrén 1994; Fahrig 1997, 2002), resulting in small suitable habitat patches surrounded by a matrix of unsuitable or suboptimal habitat. This may lead to genetic isolation of formerly connected local populations and results in decreased levels of allelic diversity and increased levels of inbreeding within local population (Leberg 1991; Wauters et al. 1994; Gerlach & Musolf 2000; Sih et al. 2000). Thus, comparing the levels of observed heterozygosity (H_o) among past and present populations may be used to monitor the relative decrease in landscape connectivity among regions resulting from habitat loss and fragmentation. During bottleneck events, the chance is higher that rare alleles are lost first. Thus, the number of alleles maintained within populations is expected to decrease rapidly during a bottleneck event, whereas the loss or rare alleles has little influence on the expected heterozygosity. Consequently, recently bottlenecked populations show an excess in heterozygosity (H_e) compared to the levels of heterozygosity expected at mutation–drift equilibrium (H_{eq}), estimated from the number of alleles maintained in the population. Cornuet and Luikart (1996) used the discrepancy between the expected heterozygosity measured from allelic frequencies (H_e) and the heterozygosity expected at equilibrium (H_{eq}) as a test to detect recent bottleneck events.

The capercaillie is a forest grouse species with its main natural range in Scandinavia and Russia. Outside of this range, the capercaillie also occurs in mountainous areas in western and central Europe (Storch 2001). The population of *T. urogallus* have declined dramatically in central Europe as a result of changes in land use, in forestry techniques and in climate (Storch 2000; 2001). The last peak in density took place at the beginning of the 20th century and, since the 1950–60s the decline of the species throughout central Europe has been pronounced and continuous. The monitoring of the species in Switzerland revealed a decline in numbers of 50% accompanied by the contraction of the distribution range of the species in the last 15 years (Marti 1986; Mollet et al. 2003). Habitat loss and fragmentation as well as human disturbances increased (Graf 2005), and these processes may further threaten the persistence of populations in the remaining habitat patches. *Tetrao urogallus* persists nowadays in five core areas in Switzerland (Mollet et al. 2003).

To estimate the levels of genetic diversity before the period of demographic decline, we analysed specimens collected before 1950. We compared these estimates with present values obtained by analysing samples from 2001–2004. Our goals were (i) to infer the genetic relationship between the past and present regional populations and (ii) to assess to what extent the decline of *T. urogallus* in the Swiss Alps since the 1950s is paralleled by of population genetic diversity and individual heterozygosity between past and present. These results should help us better understand the endangerment of *T. urogallus* in the Swiss Alps and elaborate appropriate scenarios for the management of the species.

Material and methods

Study area and data collection

Mollet et al. (2003) divided the distribution of *Tetrao urogallus* in the Swiss Alps into five core areas (inset in Fig. 1). Our study area included three of these (3, 4a and 4b in Fig. 1). We further divided the core area 4a into 4_{SZ} and 4_{SG} to better emphasize its topographic heterogeneity and renamed the core area 4b as 4_{GR} (Fig. 1). In the text, we refer to the four geographically isolated units studied as regions 3, 4_{SZ}, 4_{SG} and 4_{GR}. Habitat characteristics varied largely between the four regions both in topography and vegetation cover (Bollmann et al. 2005; Graf 2005). A study on the genetic structure of the capercaillie population in the Swiss Alps showed that regions 4_{SG} and 4_{GR} were semi-isolated units of a large population (Jacob et al. in prep), but that study did not investigate the genetic relationship among regions 3, 4_{SZ} and 4_{SG}, separated by areas of unsuitable habitat.

Because of the small size of our study area (150x100 km), we were confronted with the problem of finding museum specimens for which the exact location (valley or forest patch of origin) and the date of sampling were known. We analysed 26 museum specimens dated between 1879 and 1950 (Table 1), prior to the pronounced and continuous decline of the species in Central Europe and in Switzerland (Glutz von Blotzheim et al. 1973; Marti 1986; Mollet et al. 2003). We pooled these specimens into four past regional populations, named after the sampling regions and designated with roman numbers (III, IV_{SZ}, IV_{SG} and IV_{GR}; Fig. 1). Three specimens from region IV_{SZ} were from the same clutch (2 days old). We pooled the *T. urogallus* specimens collected during the period 2001–2004 into four extant regional populations named after the sampling regions and designated with the Arabic numbers (3, 4_{SZ}, 4_{SG} and 4_{GR}; Fig. 1). We assigned the samples collected in the four regions before the 1950s

and those collected in 2001–2004 into past and present populations in the Swiss Alps, respectively.

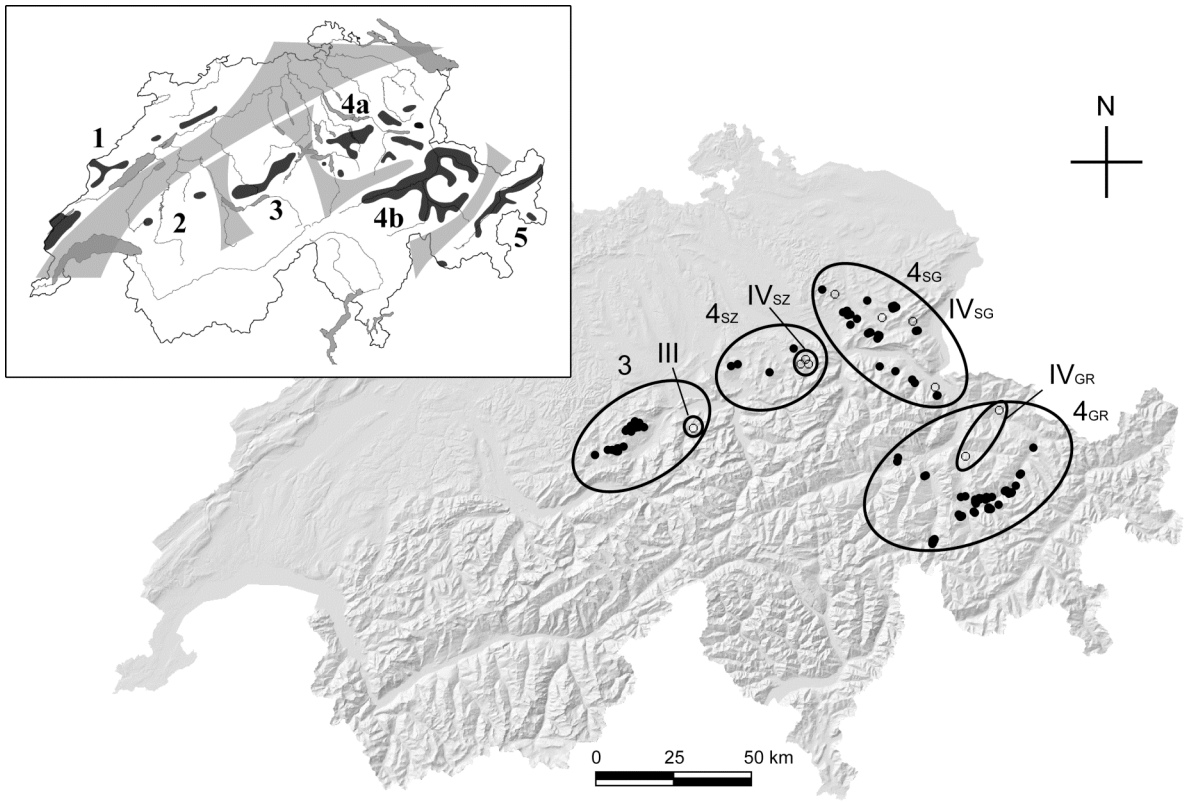

Figure 1: *Locations of the samples used in this study. The distribution of the samples collected during the years 2001–2004 (n=141) is indicated with full circles (3, 4_{SZ}, 4_{SG} and 4_{GR}). The distribution of the past samples (n=10) is indicated by open circles (III, IV_{SZ}, IV_{SG} and IV_{GR}). Inset: Distribution of* Tetrao urogallus *population in Switzerland, from Mollet et al. (2003).*

We obtained feather and footpad samples from six specimens from four different private owners and from 20 specimens from six museums in Switzerland. Three skin samples, about 2–3 mm^2, were collected from each specimen in areas where the damages to the specimens were least visible. We mostly collected skin samples from the legs, the cloacal region and from an area situated above the wings where sampling was facilitated by the absence of feathers (U. Schneppat, Bündner Naturmuseum, Chur, pers. comm.). DNA was extracted from two of the three skin samples of each of the 26 specimens. The third skin sample was going to be used only if the results of the first two samples differed. Only two

skin samples were collected on young *T. urogallus* specimens to limit damages to the specimens. We changed gloves and scalpel blades and soaked the scalpel's body and forceps for 10–15 minutes in DNA-Off® (Q-Biogene, Basel, Switzerland) solution between treating specimens to avoid cross-contamination. We did not change gloves, scalpel blades and soak forceps between collecting skin samples of the same specimen. Skin samples were stored in paper envelopes or in 2-ml plastic tubes.

Table 1: *Region, Swiss canton, location of origin, year of sampling, species to which the specimens was assigned based on genotype, number of loci amplified in each of two DNA extracts of the 26 specimens analysed (see text for details).*

Region	Canton	Location	Sampling year	Species	DNA extract #1	DNA extract #2
II	FR	Gruyère	1948	*T. urogallus*	2/12	2/12
III	NW	Wolfenschiessen	1925	*T. urogallus*	12/12	12/12
IV_{SZ}	SZ	Wägital	1882	*T. urogallus*	12/12	12/12
	SZ	Wägital	1882	*T. urogallus*	12/12	12/12
	SZ	Wägital	1882	*T. urogallus*	12/12	12/12
	SZ		1900	*T. urogallus*	4/12	4/12
	SZ	Iberg	1912	*T. urogallus*	2/12	2/12
	SZ		1929	*T. urogallus*	0/12	0/12
IV_{SG}	SG	Nesslau	ca. 1900	*T. urogallus*	11/12	11/12
	SG	Wildhaus	1912	*T. urogallus*	12/12	12/12
	SG	Nesslau	1940-50	*T. urogallus*	4/12	3/12
	ZH	Girenbad	1916	*T. urogallus*	0/12	0/12
	ZH	Bäretswil	<1950	*T. urogallus*	2/12	2/12
	SG	Vilters	1970	*T. urogallus*	12/12	12/12
	SG	Watwill	1900	*T. urogallus*	12/12	12/12
IV_{GR}	GR	Trin	1890	*T. tetrix*	11/12	0/12
	GR	Trin	1890	*T. tetrix*	11/12	11/12
	GR	Trin	1890	*T. tetrix*	9/12	9/12
	GR	Churwalden	1879	*T. urogallus*	3/12	0/12
	GR	Felsberg	1900	*T. urogallus*	4/12	3/12
	GR	Malix	1894	*T. urogallus*	0/12	6/12
	GR	Saas	1941	*T. urogallus*	3/12	2/12
	GR	Bonaduz	1959	*T. urogallus*	5/12	5/12
	GR	Reichenau	1918-42	*T. urogallus*	0/12	0/12
	GR	Prättigau	1975-80	*T. urogallus*	12/12	11/12
	GR	Churwalden	1960	*T. urogallus*	12/12	12/12

DNA was extracted with the DNA Tissue Kit (Qiagen, Hombrechtikon, Switzerland), following the manufacturer's recommendations. DNA was eluted into 2x75μl of buffer AE.

DNA extractions were initially conducted in a room dedicated to extraction of degraded and fossil DNA. Access to this room was limited to people wearing overall body protections, shoe protections and sterile gloves. Contaminations within this room were controlled by air filtering, over-pressure and soaking of the ground with bleach. One UV-illuminated hood and a pipette set were dedicated each for DNA extraction and PCR setups. We first extracted DNA from sixteen skin samples (eight specimens), using one negative extraction control per specimen to monitor cross-contaminations. We recorded no contamination from museum or extant samples. We compared the DNA yields of ancient and contemporary DNA templates, extracted from field-collected faecal samples, by amplifying mitochondrial and nuclear gene fragments by PCR. Ancient DNA templates yielded more amplification products than contemporary DNA templates, extracted from faecal samples, which suggested that the quantity and quality of the DNA extracted from the past specimens were higher than those from the contemporary samples. These results also suggested that the risk of contamination during DNA extraction was not greater using museum specimens than contemporary samples, and, therefore, that the extraction procedure set up for contemporary samples was also suitable for past specimens. We thus extracted DNA from the remaining 18 specimens (30 skin and 6 feather samples) in a dedicated room free of *T. urogallus* DNA and PCR products. Contaminations were monitored using extraction and PCR negative controls.

Our genotyping followed that described in Jacob *et al.* (submitted). We screened the allelic variation at twelve nuclear microsatellite loci divided into four PCR-multiplexes. New primer pairs for ten microsatellite loci developed for the capercaillie (Segelbacher et al. 2000) were designed based on the sequences available in Genbank (Table 2). We chose primer pairs that flank fragments in the range of 50–150bp because the probability of amplification was shown to be inversely correlated with fragment size in studies investigating degraded DNA (Gautschi 2001; Wandeler et al. 2003). We amplified two additional microsatellite loci, BG15 and BG18, developed for *Tetrao tetrix* L. (Black grouse, Piertney & Höglund 2001; Table 2). Fluorescently labelled PCR products were visualized on an ABI3100*Avant* automated sequencer (Applied Biosystems, Rotkreuz, Switzerland).

Table 2: *List of the twelve nuclear microsatellite loci used in the study. The loci were grouped into four PCR-multiplexes. For each locus, we report the number of alleles observed, the size range of the alleles and the percentage of individuals successfully genotyped.*

Multiplex	Locus	A_o	Size range	% individual genotyped
1	TuD1[a]	4	153-163	0.61
	TuD7[a]	2	94-98	0.78
	TuT4[a]	3	126-138	0.83
2	BG15[b]	3	134-142	0.43
	TuD3[a]	4	85-95	0.52
	TuT1[a]	3	128-136	0.43
3	TuD6[a]	4	162-176	0.48
	TuT3[a]	2	93-97	0.74
	TuD5[a]	11	125-155	0.43
4	TuT2[a]	5	143-163	0.48
	TuD4[a]	6	54-90	0.70
	BG18[b]	6	186-206	0.39

[a] from Segelbacher et al. (2000)
[b] from Piertney and Höglund (2001)

Statistical analyses

To estimate the genetic relatedness among regional populations, we calculated the proportion of shared alleles among individuals within regions (Bowcock et al. 1994), using the program MSA (Dieringer & Schlötterer 2003). The proportion of shared alleles was shown to perform best to infer the genetic relatedness among populations with low levels of divergence (Goldstein et al. 1995). To investigate the genetic relationship among past and present regional populations, we constructed a neighbour-joining dendrogram (Saitou & Nei 1987) using the program NEIGHBOR from the PHYLIP package (Felsenstein 2005).

To assess the levels of genetic variation within the four past and the four present regional populations, we calculated the number of alleles, A_o, private alleles, A_p, and the observed and expected heterozygosity, H_o and H_e, using GenAlEx 6 (Peakall & Smouse in press). We used FSTAT (Goudet 2001) to estimate the level of allelic richness r(g) (Petit et al. 1998), where g is the number of gene copies used in the rarefaction estimate of the allelic richness. Given the low sample size in the past regional populations, H_e and $r(g)$ were only calculated in the total past and present populations (Table 3; Leberg 2002). H_o was calculated in the eight regional populations and in the total past and present populations (Table 3). We conducted locus-by-locus comparisons to test for a decrease in genetic diversity from past to present (one-tailed paired tests). We used non-parametric tests (Wilcoxon tests) to account for

the low number of regional populations studied (n=4). All tests were computed with the R statistical package (R Development Core Team 2005).

Table 3: *Genetic characteristics of the ten* Tetrao urogallus *populations studied: number of individuals genotyped,* N, *total number of alleles observed,* A_o, *number of private alleles,* A_p, *observed heterozygosity,* H_o, *expected heterozygosity* H_e, *allelic richness, (*r*(18); Petit et al. 1998). See Fig. 1 for population codes.*

Populations	*N*	A_o	A_p	H_o	H_e	*r*(18)
III	1	16	0	0.778	-	-
IV_{SZ}	3	24	1	0.630	-	-
IV_{SG}	4	32	0	0.750	-	-
IV_{GR}	2	24	1	0.889	-	-
Past	10	40	3	0.743	0.645	4.331
3	31	38	1	0.462	-	-
4_{SZ}	4	28	1	0.454	-	-
4_{SG}	52	50	4	0.643	-	-
4_{GR}	54	48	2	0.649	-	-
Present	141	58	21	0.552	0.644	4.210

The program BOTTLENECK (Piry et al. 1999) was used to test for a recent bottleneck event. We used the two-phase mutation model (TPM, Dirienzo et al. 1994), assuming 95% single-step and 5% multiple-step mutations as recommended by Piry et al. (1999). We computed the distribution of the heterozygosity expected under the assumption of mutation–drift equilibrium at each locus, from the number of alleles and the sample size, using 1000 replicates. Given the number of loci screened, we used the Wilcoxon sign-rank test to determine if significant numbers of loci showed heterozygosity excess, as recommended by the authors. We conducted the test in the three present regional populations in which a minimum of 30 individuals were sampled, *i.e.* 3, 4_{SG} and 4_{GR}.

Results

DNA was successfully amplified at 9–12 microsatellite loci from 25 out of 52 skin samples analysed (13 individuals out of 26; 50%). Independent replicates of DNA extraction and amplification resulted in the same multi-locus genotype at 11–12 loci in ten individuals and at nine loci in one individual. In one museum specimen, only one DNA extract out of two

replicates could be amplified (Table 1). We detected no cross-contaminations between past specimens or from extant *T. urogallus* DNA. Three young specimens (2 days old), labelled as *T. urogallus*, showed allele sizes characteristic for the sister species, *T. tetrix* (Table 1).

The proportion of positive PCRs largely varied among loci and decreased exponentially with increasing length of the target fragments ($y=-0.64\log(x)+1.9$, $R^2=0.32$; Table 2). The proportion of successful PCRs ranged from 0.52 to 0.78 for fragments in the range 50–100 bp, 0.43 to 0.83 for fragments in the range 120–150 bp and the lowest success rate (0.39) was observed for the largest fragments, around 200 bp (Table 2). All loci were polymorphic, showing 2–11 alleles (mean=4.4; Table 2). One private allele each was found in IV_{SZ} and IV_{GR} and one allele was found in all past individuals but not in the total present population (Table 3). A total of 21 alleles were found only in the total present population, the number of private alleles per present regional population ranged from 0 to 4. Jacob et al. (submitted) showed that three loci, TuD6, TuD4, TuT1 had one or more null alleles and we therefore used the same set of nine microsatellite loci when comparing genetic characteristics and calculating genetic distances between individuals.

The neighbour-joining dendrogram (Fig. 2) showed that regional populations tended to cluster by sampling period (before 1950 *vs.* 2001–2004) rather than by region of sampling.

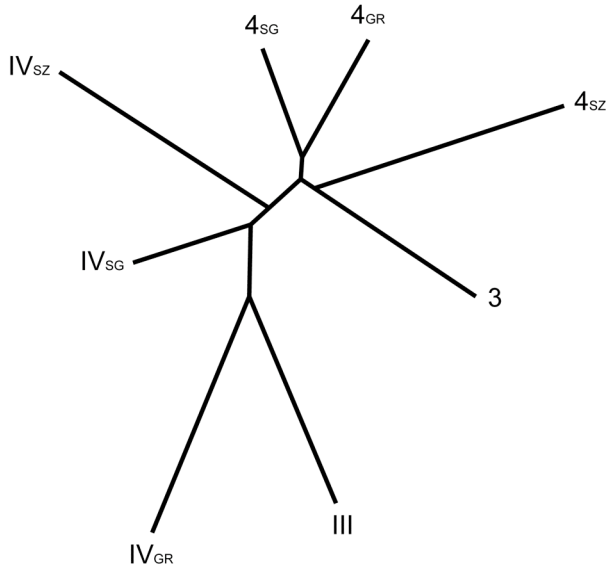

Figure 2: *Neighbour-joining dendrogram (Saitou and Nei, 1990), based on the proportion of alleles shared among populations (Bowcock et al. 1994), showing the genetic relationship among the past (III, IV_{SZ}, IV_{SG} and IV_{GR}) and present (3, 4_{SZ}, 4_{SG} and 4_{GR}) regional populations.*

We found a significant decrease in H_o between the total past and present populations (Wilcoxon, p=0.006). Pairwise comparisons among past and present regional populations also revealed a significantly lower level of H_o in 4_{GR} than in IV_{GR} (Wilcoxon, p=0.01) and a marginally significant decrease over time in H_o in the three other regions (Wilcoxon, 0.054<p<0.064). No significant decreases in H_e (Wilcoxon, p=0.89) and *r(g)* (Wilcoxon, p=0.5) were found between the total past and present populations.

We detected no significant differences in the levels of expected heterozygosity calculated from alleles frequencies (H_e) and the level of heterozygosity expected at equilibrium calculated from he number of alleles (H_{eq}) in regional populations 3, 4_{SG} and 4_{GR} (Table 4).

Population	H_e	H_{eq}	p-value $H_e>H_{eq}$	p-value $H_e<H_{eq}$	p-value $H_e\neq H_{eq}$
3	0.51	0.51	0.41	0.63	0.82
4_{SG}	0.64	0.66	0.46	0.59	0.91
4_{GR}	0.66	0.64	0.82	0.21	0.43

Table 4: *Summary table of the test to detect a recent bottleneck event in three present populations, 3, 4_{SG} and 4_{GR}: levels of heterozygosity calculated from allele frequencies (H_e), heterozygosity expected under mutation–drift equilibrium (H_{eq}), p-values of Wilcoxon rank tests for the null hypothesis of heterozygosity excess ($H_e>H_{eq}$), heterozygosity deficiency ($H_e<H_{eq}$) and difference between the two parameters ($H_e\neq H_{eq}$).*

Discussion

Our study demonstrates that museums specimens of *Tetrao urogallus* can be used as a source of DNA to investigate the genetic composition of the past capercaillie population of the Swiss Alps. Additionally, it shows that nuclear microsatellite loci can be used to assess the taxonomic status of museum specimens. These are two important pre-requisites that allow one to evaluate historical processes more directly than by inference based on current population screening.

Our results suggest that the distribution of *T. urogallus* in the Swiss Alps formed a network of regional populations connected by gene flow at least until the 1950s. These regional populations subsequently became more isolated by habitat fragmentation and loss between them and at their margin. Johnson et al. (2004) found a similar change in genetic structure over time with small differences between original regional populations in the related species, *Tympanuchus cupido* L.. These empirical observations are consistent with predictions from theoretical and simulation studies, showing that population fragmentation leads to genetic differentiation between populations, whereas expected heterozygosity, H_e, of individuals can remain high (Varvio et al. 1986; Keyghobadi et al. 2005). Thus, fragmentation of large populations into isolated patches may result in discrepancies between the estimates of genetic differentiation between patches measured by F_{ST} and genetic diversity within patches measured by H_e (Goodman et al. 2001; Keyghobadi et al. 2005). Jacob et al. (in prep) found significant levels of genetic differentiation among regional populations 3, 4_{SG} and 4_{GR}, although regional population 4_{SG} and 4_{GR} showed evidence for genetic connectivity. In the present study, we detected significantly lower levels of observed heterozygosity, H_o, in the present than in the past total population but we observed no significant decline in expected heterozygosity, H_e, and allelic richness, *r(g)* (Petit et al. 1998), which is consistent with the predictions.

We detected private alleles in past and present regional populations. One allele ($TuD5_{125}$) was found in all past regional populations, but was not found in the present regional populations, despite of the larger sample size in the present than in the past regional populations. The mutation of this allele in all the present regional populations in a period of 50–100 years seems unlikely. These results suggest that a shift in the local allele occurrence and frequencies took place between the two time periods studied.

Marti (1986) and Mollet et al. (2003) illustrated the decline in distribution and in number of *T. urogallus* in Switzerland during the last 30 years. Graf (2005) showed that habitat degradation impacted the viability of local populations in the capercaillie in the Swiss Alps. Local populations occupying small habitat patches showed a greater probability of extinction due to small population size and reduced connectivity (Bollmann et al. submitted). Empirical studies showed that genetically isolated populations may experience inbreeding depression, as shown in a related species, *Tympanuchus cupido* (Westemeier et al. 1998) and in *Vipera berus* L. (Madsen et al. 1996). However, no study so far investigated the impact of inbreeding depression on present *T. urogallus* regional populations. Translocation of

individuals to increase the gene flow between populations may decrease the impact of inbreeding depression (Hedrick 1995; Westemeier et al. 1998; Madsen et al. 1999; Madsen et al. 2004). This has been demonstrated in several endangered plant species (Fischer & Matthies 1997; Paschke et al. 2002). Although the results of plant studies may not be directly applicable for the management of animal species, where only few cases of outbreeding depression have been recognized, they illustrate well the potential threats to local population persistence in endangered species (Frankham 1995). At the same time, translocation of individuals from distant populations bears the risk of spreading new diseases and pathogens, which may further jeopardize the viability of the population under management. In contrast, experimental studies have shown that, in a constellation of partially inbred populations, immigrant genome tend to spread due to hybrid vigour (heterosis), and that this effect may have a beneficial effect on individuals' fitness within populations (Spielman & Frankham 1992; Saccheri & Brakefield 2002). Although these studies were conducted on fruit flies (*Drosophila melanogaster* Meigen) and butterflies (*Bicyclus anynana* Butler) bred under laboratory conditions, they showed that heterosis may have a major influence on the evolution of naturally of artificially fragmented and partially inbred populations, like the capercaillie. We therefore recommend that management actions should focus on restoring the landscape and genetic connectivity among regional populations to promote the sustainability of the capercaillie population in the Swiss Alps.

Studying the genetic relationship among the *T. urogallus* population in the Swiss Alps, in the Jura (core area 1 in inset Fig. 1) and in southern Germany, northern Italy and Austria may provide additional evidence to infer the status of the capercaillie population in Switzerland. Such studies are required to determine management units and priority areas for the conservation of the capercaillie in the Alps.

Because of the small size of the study area (150x100 km), we were confronted with the problem of finding sufficient numbers of museum specimens for which the exact location (valley or forest of origin) and the date of sampling were known. The low number of museum specimens analysed consequently reduced our power to assess the dynamic of the genetic diversity between the two time periods studied. However, sampling density should have no impact on the estimation of the levels of H_o, and we could still identify a particular loss of a formerly widespread allele. Thus, our results suggest that level of genetic diversity maintained in the regional populations has been impacted by the decline of the *T. urogallus*

population in the Swiss Alps, but we missed statistical power to assess the magnitude of the decrease.

From the results of our study, we conclude that past specimens can be used to investigate the temporal dynamics of the genetic variability at a restricted geographical area. The laboratory procedures required to work with degraded DNA have been established and are nowadays used in routine in many laboratories. Museum and private collections comprise considerable numbers of specimens and provide the opportunity to conduct genetic analyses on a wide range of common, declining, rare and even extinct species or subspecies. However, we point out that species misidentification in museum and other collections could be a source of error when using past specimens in molecular genetic studies. We therefore recommend that only museum specimens with precise records be used in molecular studies, or else taxonomic confirmation is required within the genetic analysis.

References

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos*, **71**, 355-366.

Bollmann K, Graf RF, Suter W (submitted) Assessing the role of patch area, isolation, and connectivity for predicting capercaillie occurrence in the Alps.

Bollmann K, Weibel P, Graf RF (2005) An analysis of central Alpine capercaillie spring habitat at the forest stand scale. *Forest. Ecol. Manag.*, **215**, 307-318.

Bouzat JL, Cheng HH, Lewin HA, Westemeier RL, Brawn JD, Paige KN (1998a) Genetic evaluation of a demographic bottleneck in the Greater Prairie Chicken. *Conserv. Biol.*, **12**, 836-843.

Bouzat JL, Lewin HA, Paige KN (1998b) The ghost of genetic diversity past: Historical DNA analysis of the greater prairie chicken. *Am. Nat.*, **152**, 1-6.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455-457.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001-2014.

Dieringer D, Schlötterer C (2003) MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes*, **3**, 167-169.

Dirienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human-populations. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3166-3170.

Ellegren H (1994) Genomic DNA from museum birds feathers. In: *Ancient DNA* (ed. Hermann B HS), pp. 211-217. Springer, New York.

Fahrig L (1997) Relative effects of habitat loss and fragmentation on population extinction. *J. Wildlife Manage.*, **61**, 603-610.

Fahrig L (2002) Effect of habitat fragmentation on the extinction threshold: A synthesis. *Ecol. Applic.*, **12**, 346-353.

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6, Department of Genome Sciences, University of Washington, Seattle.

Fischer M, Matthies D (1997) Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). *Am. J. Bot.*, **84**, 1685-1692.

Frankham R (1995) Conservation genetics. *Annu. Rev. Genet.*, **29**, 305-327.

Frankham R (2005) Genetics and extinction. *Biol. Cons.*, **126**, 131-140.

Gautschi B (2001) Conservation genetics of the Bearded Vulture (*Gypaetus barbatus* L.). PhD Thesis, University of Zurich, Zurich.

Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conserv. Biol.*, **14**, 1066-1074.

Glutz von Blotzheim UN, Bauer KM, Bezzel E (1973) *Tetrao urogallus*. In: *Handbuch der Vögel Mitteleuropas*, pp. 172-225, Akademische Verlagsgesellschaft, Frankfurt am Main.

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. *Genetics*, **139**, 463-471.

Goodman SJ, Tamate HB, Wilson R, Nagata J, Tatsuzawa S, Swanson GM, Pemberton JM, McCullough DR (2001) Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. *Mol. Ecol.*, **10**, 1357-1370.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices. University of Lausanne, Switzerland.

Graf RF (2005) Analysis of capercaillie habitat at the landscape scale using aerial photographs and GIS. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Zurich.

Groombridge JJ, Jones CG, Brudford MW, Nichols RA (2000) 'Ghost' alleles of the Mauritius kestrel. *Nature*, **403**, 616.

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.*, **11**, 2467-2474.

Hedrick PW (1995) Gene flow and genetic restoration - the Florida Panther as a case-study. *Conserv. Biol.*, **9**, 996-1007.

Jacob G, Bollmann K, Schmid B, Gugerli F (in prep) Landscape barriers induce genetic structuring in the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps - implications for the management of the species.

Jacob G, Debrunner R, Gugerli F, Schmid B, Bollmann K (submitted) Estimating the population census sizes of capercaillie (*Tetrao urogallus* L., Aves) in the Swiss Alps from single-occasion genetic capture–recapture data. *Cons. Genet.*

Johnson JA, Bellinger MR, Toepfer JE, Dunn P (2004) Temporal changes in allele frequencies and low effective population size in greater prairie-chicken. *Mol. Ecol.*, **13**, 2617-2630.

Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Mol. Ecol.*, **12**, 3335-3347.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends Ecol. Evol.*, **17**, 230-241.

Keyghobadi N, Roland J, Matter SF, Strobeck C (2005) Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. *Proc. R. Soc. London Ser. B*, **272**, 553-560.

Lande R (1994) Risk of population extinction from fixation of new deleterious mutations. *Evolution*, **48**, 1460-1469.

Leberg PL (1991) Influence of fragmentation and bottlenecks on genetic divergence of Wild Turkey populations. *Conserv. Biol.*, **5**, 522-530.

Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. *Mol. Ecol.*, **11**, 2445-2449.

Madsen T, Shine R, Olsson M, Wittzell H (1999) Conservation biology - restoration of an inbred adder population. *Nature*, **402**, 34-35.

Madsen T, Stille B, Shine R (1996) Inbreeding depression in an isolated population of adder *Vipera berus*. *Biol. Cons.*, **75**, 113-118.

Madsen T, Ujvari B, Olsson M (2004) Novel genes continue to enhance population growth in adders (*Vipera berus*). *Biol. Cons.*, **120**, 145-147.

Marti C (1986) Verbreitung und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz. *Ornithol. Beob.*, **83**, 67-70.

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): Implications for conservation. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 4334-4339.

Miller LM, Kapuscinski AR (1997) Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics*, **147**, 1249-1258.

Mollet P, Badilatti B, Bollmann K, Graf RF, Hess R, Jenny H, Mulhauser B, Perrenoud A, Rudmann F, Sachot S, Studer J (2003) Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. *Ornithol. Beob.*, **100**, 67-86.

Nielsen EE, Hansen MM, Loeschcke V (1997) Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: A comparison of genetic composition over 60 years. *Mol. Ecol.*, **6**, 487-492.

Paschke M, Abs C, Schmid B (2002) Relationship between population size, allozyme variation, and plant performance in the narrow endemic Cochlearia bavarica. *Cons. Genet.*, **3**, 131-144.

Peakall ROD, Smouse PE (in press) GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes.*

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, **12**, 844-855.

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Mol. Ecol. Notes*, **1**, 303-304.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.*, **90**, 502-503.

R Development Core Team (2005) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Saccheri IJ, Brakefield PM (2002) Rapid spread of immigrant genomes into inbred populations. *Proc. R. Soc. London Ser. B*, **269**, 1073-1078.

Saitou N, Nei M (1987) The neighbor-joining method - A new method for reconstructing phylogenetic trees. *Molecular Biology & Evolution*, **4**, 406-425.

Segelbacher G, Paxton RJ, Steinbruck G, Trontelj P, Storch I (2000) Characterization of microsatellites in capercaillie *Tetrao urogallus*. *Mol. Ecol.*, **9**, 1934-1935.

Sih A, Jonsson BG, Luikart G (2000) Habitat loss: ecological, evolutionary and genetic consequences. *Trends Ecol. Evol.*, **15**, 132-134.

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 15261-15264.

Spielman D, Frankham R (1992) Modeling problems in conservation genetics using captive *Drosophila* populations - Improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. *Zoo Biol.*, **11**, 343-351.

Storch I (2000) Grouse Status Survey and Conservation Action Plan 2000-2004. (ed. Group WBSGS), p. 112 pp. IUCN, Gland, Switzerland and Cambridge, UK and the World Pheasant Association, Reading, UK.

Storch I (2001) *Tetrao urogallus* Capercaillie. *BWP Update*, **3**, 1-24.

Varvio SL, Chakraborty R, Nei M (1986) Genetic variation in subdivided populations and conservation genetics. *Heredity*, **57**, 189-198.

Wandeler P, Smith S, Morin PA, Pettifor RA, Funk SM (2003) Patterns of nuclear DNA degeneration over time - a case study in historic teeth samples. *Mol. Ecol.*, **12**, 1087-1093.

Wauters LA, Hutchinson Y, Parkin DT, Dhondt AA (1994) The effects of habitat fragmentation on demography and on the loss of genetic-variation in the red squirrel. *Proc. R. Soc. London Ser. B*, **255**, 107-111.

Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695-1698.

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Chapter 5

Feasibility of individual-based genetic monitoring of rare and elusive species – Preliminary results from the capercaillie (*Tetrao urogallus*) in the Swiss Alps

Gwenaël Jacob[1,2]*, Roland Graf[1,3], Felix Gugerli[1], Bernhard Schmid[2] and Kurt Bollmann[1]

[1] Swiss Federal Research Institute WSL, Birmensdorf

[2] Institute of Environmental Sciences, University of Zurich

[3] present address: Department of Ecological Modelling, Centre for Environmental Research Leipzig-Halle, Germany

Abstract

In the last decade, the range of genetic studies of animal populations based on non-invasive sampling techniques has dramatically increased. In combination with highly polymorphic markers such as microsatellites, non-invasive genetic studies have filled gaps in behavioural ecology that were not accessible by conventional field studies. In the present study, we assessed the feasibility of individual-based monitoring of the capercaillie, *Tetrao urogallus* L., in the Swiss Alps using non-invasive sampling and genetic markers. Our goals were (i) to detect individual dispersal within and among habitat patches, (ii) to estimate individual habitat use and (iii) to reconstruct parentage relationships. We observed the largest dispersal distances between leks in one region where capercaillie habitat consists of suitable

forest patches separated by agricultural landscapes. This result suggests that capercaillie inhabiting mosaic landscapes tended to disperse across larger distances than those inhabiting large and contiguous habitat patches. Estimates of habitat use did not differ among the three regions studied or between males and females. Parentage analyses were conducted in three local populations, in which sampling was most intensive. The probability to assign putative parents to offsprings was smaller than 50% in all but one local population which had the lowest probability of identity between two individuals ($P(ID)$=1.75 x 10^{-6}). In this local population, three offspring were assigned each to unique parent pairs whereas two were assigned to the same parent pair. Our study demonstrates that individual-based genetic monitoring is feasible in the capercaillie. Moreover, our results suggest that genetic analyses of non-invasive samples might be of value to study breeding success in lekking species like the capercaillie, for which a large proportion of the population can be sampled with limited effort during the breeding season.

Keywords – non-invasive sampling, microsatellite, home range, dispersal, habitat model, individual-based monitoring

Introduction

Molecular markers have been successfully applied to study the levels of genetic variability and genetic divergence among populations or species and to investigate evolutionary processes. However, parameter estimates from population genetic studies mostly reflect the long-term evolution of the population or species and thus may be of limited relevance for ecological studies or conservation. Owing to the availability of highly variable genetic markers, individual-based studies are nowadays feasible that allow investigating individual behaviour or dispersal (Palsbøll 1999).

Owing to recent improvements in the techniques of DNA preservation, extraction and amplification, non-invasive samples that yield minute amounts of degraded DNA have been used as sources of DNA for molecular studies (Woodruff 1993; Palsbøll et al. 1997; Taberlet et al. 1997). The PCR allows using picograms of DNA as template for amplification, but bears the risk that one or both of the two alleles of a heterozygous individual goes undetected (allelic dropout, ADO) or that false alleles (FA) result from artefacts induced by the polymerase (Taberlet et al. 1996). Additional genotyping errors may result from non-amplifying alleles (null alleles, Pemberton et al. 1995) or amplification of non-target

fragments (Bradley & Vigilant 2002). Genotyping errors and reduced power to distinguish among individuals if insufficient numbers of molecular markers are screened may result in mistakes in individual identification (Taberlet & Luikart 1999; Waits et al. 2001). To control for genotyping errors, precautious laboratory procedures, including, when possible, multiple extractions and PCRs of the same samples, have been recommended (Navidi et al. 1992; Taberlet et al. 1996).

Identification of individuals based on their multi-locus genotypes has been used to estimate population census sizes from faeces and hair samples (Kohn et al. 1999; Mowat & Paetkau 2002; Eggert et al. 2003; Wilson et al. 2003; Frantz et al. 2004) and sloughed skin samples (Palsbøll et al. 1997). Theoretical studies investigated the effects of genotyping and individual identification errors (Mills et al. 2000; Petit & Valière in press) and contributed to a better understanding of the factors affecting the accuracy of population size estimates.

Genetic tags have been successfully used to assess the degree of genetic relatedness among individuals within groups (Amos et al. 1993; Morin et al. 1993) and to investigate mating systems (Gavin et al. 1998; Garnier et al. 2001; Hampton et al. 2004; Rudnick et al. 2005). Assuming that each individual can be identified by a unique genetic tag, assigning non-invasive samples collected at different locations may allow investigating habitat use. Given that population dynamic parameters and individual dispersal behaviour can be inferred through non-invasive genetic methods, they may be of great value to elaborate management plans for rare and endangered species.

The capercaillie (*Tetrao urogallus* L.) is a grouse species with its main distribution range in the taiga-like forests from Norway to Siberia. The species also occurs in the mountainous areas covered with coniferous uneven-aged forests in Western and Central Europe (Storch 2001). From late winter to mid spring, capercaillie males converge on lekking sites, usually situated in old-growth forest, on forest clearings, mires or at elevated sites. During the breeding season, capercaillie males show a territorial behaviour. Males 4 years old and older defend the smallest territories within 1 km from the lek, whereas 1-2 years old males occupy large home ranges and do not show territorial behaviour (Wegge & Larsen 1987; Storch 1997). Females usually stay further away from the leks than males and visit one or several leks during brief periods (Wegge & Rolstad 1986; Storch 1997). The annual home range of females is *c*. 550 ha in the Bavarian Alps (Storch 1995), although females visiting several leks may have larger annual home ranges (Storch 1997). Studies of capercaillie dispersal are reviewed in Storch (2001). Maximum observed seasonal movements of adult

capercaillie in central Europe were 9 km in the German Alps and 8 km in the Pyrenees. Radio-tracking studies showed that males are mostly philopatric and females tend to disperse 5-10 km, but larger distances were also observed.

In the last decades, changes in sylvicultural practices and increasing human disturbances have resulted in the fragmentation of capercaillie habitat in Scandinavia (Helle et al. 1994) and Central Europe, especially in lowland areas (Storch 2000, 2001). In Switzerland, the capercaillie population experienced a rapid decline in numbers and distribution range during the last 30 years (Marti 1986; Mollet et al. 2003), and the species is nowadays restricted to large mountainous forests, geographically isolated from each other by large distances of unsuitable habitat and topographical barriers (Mollet et al. 2003; Jacob et al. in prep). Graf (2005) used landscape and forest variables and a validation procedure by presence/absence data to model capercaillie habitat in the Swiss Alps. Based on the areas of the suitable habitat patches and the geographic distances between them, Graf et al. (2004) identified key areas for the conservation of *T. urogallus* in the Swiss Alps. Within regions, local populations inhabiting these patches were not genetically differentiated (unpublished data), which may indicate that habitat patches were connected by gene flow. However, no quantitative or qualitative estimates of individual dispersal are so far available. Census size estimates of capercaillie local populations from genetic analyses of non-invasive samples suggest that the true number of individuals in some local populations has been underestimated during the field-monitoring program of the species (Debrunner et al. 2005; Jacob et al. submitted). Yet, estimates of breeding success per individual, juvenile and adult mortality within and dispersal between habitat patches would be required to calibrate population viability models and to elaborate conservation measures at the habitat patch level.

In the present study, we address the feasibility of individual-based genetic studies (i) to monitor dispersal within and between habitat patches, (ii) to estimate individual home ranges of the capercaillie in the Swiss Alps and (iii) to reconstruct candidate parent–offspring relationships within local populations.

Material and methods

Study area and sampling

We conducted our sampling of capercaillie individuals in five regions situated in areas where the habitat quality and population densities were highest (A–D and S, Fig. 1). In region

A, the capercaillie is distributed over large and continuous areas of suitable forest. In region S, the capercaillie is distributed into several large and small habitat patches separated by valleys. In region B, it persists in isolated forest patches surrounded by agricultural landscapes. The situation is intermediate in regions C and D, where patches of suitable habitat are surrounded by a matrix of suboptimal forest along the valley slopes.

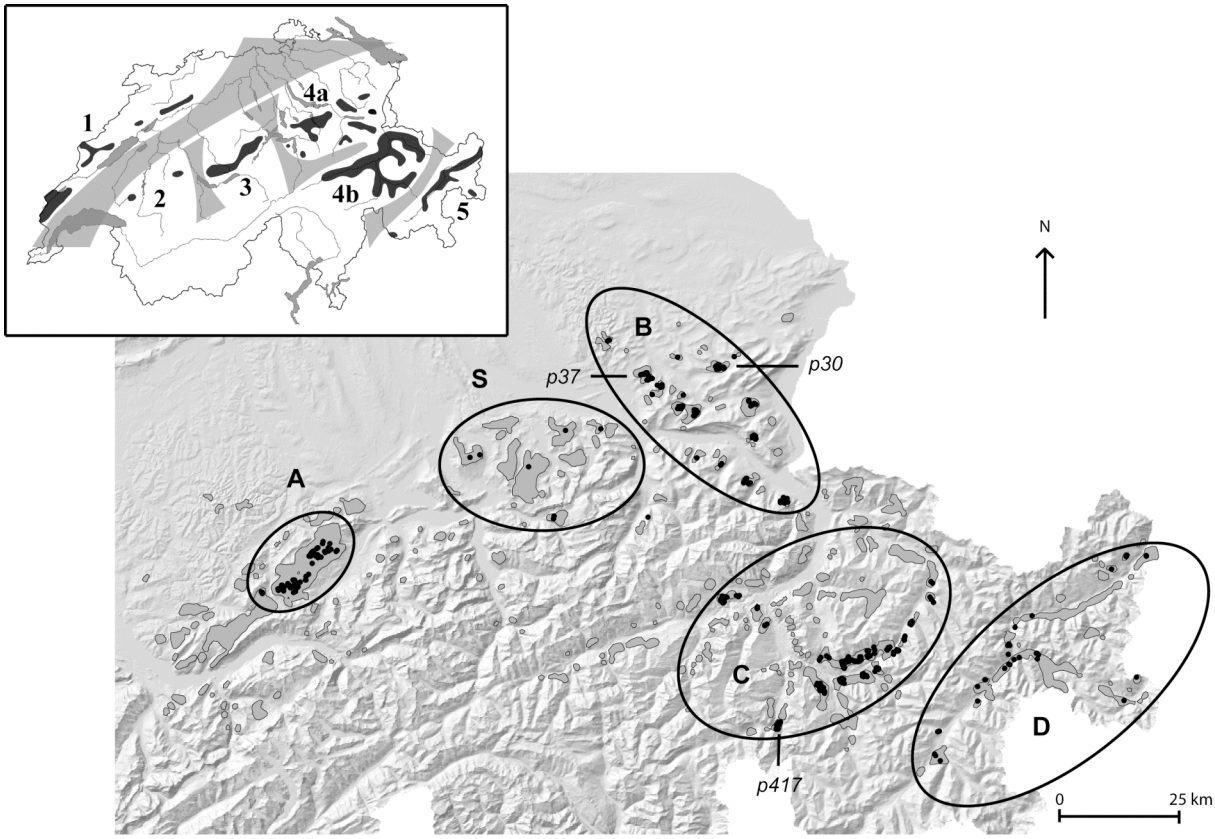

Figure 1: *Distribution of the samples collected during the period 2001–2004. Grey shadings indicate suitable habitat patches for the capercaillie assessed by a habitat model for the species (Graf 2005), data provided by R. Graf. Inset: distribution range of the capercaillie in Switzerland from Mollet et al. (2003). Our study area overlapped part of region 3 and regions 4a, 4b and 5.*

When searching for samples, we concentrated on key elements of capercaillie winter and spring habitat: feeding and roosting trees, rest sites, low-branched trees, lek areas, internal forest edge. This approach was used because the capercaillie has as strong preference for few structural and nutritional forest components during winter (Storch 2001). Sampling was

conducted in early spring, *i.e.* during the breeding season, on a single occasion in most sampling locations. We collected faecal samples and feathers separated by approximately 250 m, but we deviated from this rule if we had the opportunity to collect samples in better condition, *i.e.* likely to provide a better DNA source for genetic analyses. Given that the sizes and shapes of faeces samples or the coloration patterns of feathers samples differ between males and females, we collected samples following the procedure described above and distinguishing between sexes. Owing to the lekking behaviour of the capercaillie, we expected the highest densities of individuals in the vicinity of the lekking arenas, and we therefore collected samples separated each other by short distances.

Different sampling regimes were applied in three of the forest patches studied. Multiple samplings were conducted in habitat patch 30 (Fig. 1). A local capercaillie expert first collected faecal samples during regular visits to the lek area, before a collective sampling was organised in this forest patch. Multiple samplings were also conducted in habitat patch 37 (Fig. 1), where different people collected faecal samples at different dates. Sampling was conducted on a single occasion in habitat patch 417, where three people collected large numbers of faecal samples.

Faecal samples were collected in plastic tubes and air-dried or dried on silica gel, and feather samples were collected in paper envelops. Samples were stored at -20°C or at room temperature until extraction.

DNA extraction and genotyping

The procedures for DNA extraction from faecal and feather samples and for genotyping are described in detail in Jacob et al. (submitted). Negative controls were included to monitor cross-sample contaminations during extraction and genotyping. Four PCR-multiplexes of three primer pairs were designed to amplify twelve polymorphic nuclear microsatellite loci, ten developed for *T. urogallus* (Segelbacher) and two for *T. tetrix* (Piertney & Höglund 2001). The amplification products were visualized on an ABI3100Avant automated sequencer (Applied Biosystems, Rotkreuz, Switzerland) and the allele lengths were coded using Genescan® 3.1 and Genotyper® 2.5 softwares (Applied Biosystems, Rotkreuz, Switzerland). We recorded loci in which the same allele for homozygous individuals or the same two alleles for heterozygous individuals were recorded on three out of four electrophoretic profiles. Loci that did not meet these criteria were amplified in four

additional PCRs. We then recorded those loci in which the same allele for homozygous individuals or the same two alleles for heterozygous individuals were recorded on three out of eight electrophoretic profiles. Loci that did not meet these criteria were considered as missing values in the particular individual.

Monitoring individual movement and home ranges

We refer to the repeated occurrence of the same genotype as movements. Thus, estimates of movements could be as low as 0 m if two or more samples assigned to the same genotype had been collected at the same position. Assuming that samples were collected along a single track, we started from one end of the track and calculated the straight distance to the closest point. We recorded all distances between samples as different movement steps per individual. We estimated habitat use as the minimum area of a minimum convex polygon (MCP) including the locations of all the samples assigned to the same individual using the Convex-Hulls-Around-Points extension (Jenness 2005) for Arcview 3.2 (ESRI). We studied if we could detect a difference in the mean, maximum and minimum dispersal distances and habitat use between males and females using two-sample Wilcoxon tests, calculated with the R statistical package (R Development Core Team 2005).

We refer to movement steps and MCP as estimates of dispersal and habitat use of individuals, respectively. Nevertheless, we are aware that our estimates of dispersal distances and habitat use are tentative and do not intend to represent the dispersal capacity and effective use of the habitat by the individuals.

Parentage analyses

We conducted parentage analyses in those three habitat patches (habitat patches 30, 37 and 417) where intensive sampling was conducted, *i.e.* where the probability was highest that a large proportion of the individuals of these local populations were sampled. We used the program Gimlet (Valière 2002) to calculate the probability of identity among genotypes within local populations. The program Parente (Cercueil et al. 2002) allowed us to reconstruct possible parent–offspring relationships based on allelic similarities between the genotypes of putative parents and their offspring. The program makes use of the allele frequencies, calculated over all individuals, and an estimate of the population census size, provided by the user, to calculate the probability of the putative parents being the true parents. Genotypes that could not be included within a father–offspring or mother–offspring relationship, *i.e.*

unrelated individuals, were recorded in a separate file. We assessed, in each local population, the number of candidate parent–offspring relationships and their associated probability, and the number of unrelated genotypes.

Results

Individual genotyping

We could amplify one or more loci from 351 DNA extracts (66%), of which 279 were assigned to *T. urogallus*, 28 to *T. tetrix* and eight to *B. bonasia* based on the species-specific allele size ranges observed at BG15 and BG18. Five genotypes showed allele sizes specific for both capercaillie and black grouse at BG15 and BG18, which indicated hybridization events between *T. urogallus* and *T. tetrix*. Thirty-one samples did not amplify at BG15 and BG18 and were excluded from our analyses. Of the 279 samples assigned to capercaillie, 244 samples successfully amplified at eight to twelve loci. Those 244 DNA extracts grouped into 151 unique allelic combinations at those loci that were amplified. All unique allelic combinations differed by at least two alleles at the same or at different loci.

Individual movements and home ranges

In total, 64 individuals were sampled at more than one position, 62 movements were recorded within patches (data not shown) and 2 were movements between patches. We found several samples assigned to individual Tu148 in habitat patch 60, and we collected a sample assigned to this individual one month later in habitat patch 43 at a straight distance of 5.6 km (Fig. 2, Table 1). One sample assigned to a male, Tu156, was collected each in habitat patch 37 and the next day in habitat patch 43, situated at a straight distance of 3.5 km. We could calculate the MCP of 14 individuals for which more than two samples were collected and genotyped (Table 1). In region A, estimates of habitat use ranged from 2.42–25.48 ha. A female, for which we recorded a movement of 1.5 km, showed the largest habitat use (Table 1). In region B, estimates of habitat use within patch ranged from 1.55–37.56 ha and was largest in a male for which we recorded a movement of 0.9 km. Tu148 showed the largest habitat use in the present study, 82.11 ha, owing to an inter-patch movement of 5.6 km (Table 1). Estimates of habitat use ranged from 0.04–12.92 ha in region C. A female for which we recorded a movement of 1.8 km showed the largest habitat use (Table 1). We found no differences in habitat use between regions and between males and females (Wilcoxon, $p>0.05$).

Table 1: *List of the 14 individuals for which habitat use was estimated. We report the sex of the individuals, the movement steps recorded and the minimum convex polygon (MCP) that comprised the locations where the individual genotype was found. The number of locations used to estimate MCP is indicated in bracket. We also report the ID and the area of the habitat patches in which the samples were collected (data from Graf, 2005). In the case of inter-patch dispersal (Tu148), we report the ID of the patch in which the genotype was first found.*

Ind.	Sex	Occurrences	Movements (m)	Habitat use (ha)	Patch ID	Patch area (ha)	Sampling region
Tu059	f	7.5.2002		2.74 (3)	p248	12261	A
		9.5.2003	230				
		10.5.2002	285				
Tu061	?	27.5.2003	382	25.48 (3)	p248	12261	
			1517				
Tu062	m	28.5.2003	89	2.42 (4)	p248	12261	
			94				
			272				
Tu134	f	28.3.2003	145	22.15 (3)	p37	1076	B
			368				
Tu136	m	19.3.2003	132	3.28 (4)	p37	1076	
			134				
			472				
Tu144	?	26.3.2003	237	3.33 (3)	p60	1624	
			288				
Tu148	?	26.3.2003	328	82.11 (4)	p60	1624	
			502				
		24.4.2003	5597				
Tu121	m	10.3.2003		37.56 (5)	p127	121	
		11.3.2003	629				
			130				
			69				
			909				
Tu125	m	18.3.2003		0.04 (3)	p272	887	
		26.4.2003	200				
			4				
Tu156	?	28.3.2003					
		29.3.2003	3486				
Tu050	?	17.4.2002	143	1.55 (3)	p244	701	C
			278				
Tu006	m	30.5.2002	131	0.51 (3)	p396	1996	
		30.5.2002	270				
Tu030	?	11.4.2002	493	1.96 (3)	p339	3901	
			235				
Tu039	f	22.4.2002		12.92 (3)	p339	3901	
		24.4.2002	982				
		25.4.2002	1830				
Tu045	m	2.5.2002	363	2.74 (3)	p339	3901	
			180				

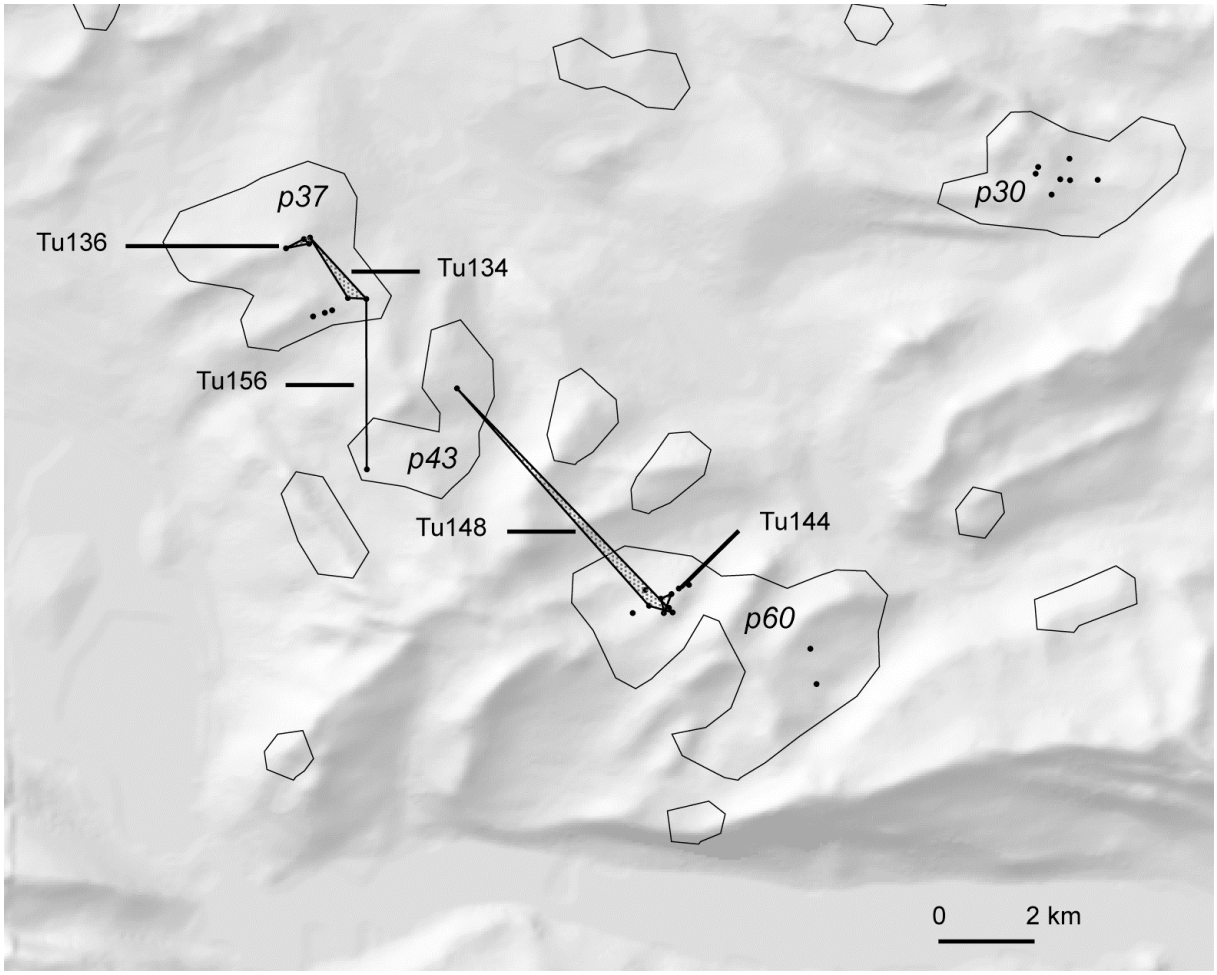

Figure 2: *Illustration of four home ranges inferred from the minimum convex polygon including all the locations of non-invasive samples assigned to an individual. The dispersal event of individuals Tu156 is also illustrated. Patch IDs are indicated in italic.*

Parentage analysis

In habitat patch 30, the probability of two individuals sharing the same genotype was $P(ID)=1.75 \times 10^{-6}$. In this habitat patch, we found two possible parent–offspring relationships, although the candidate parent relationships had a very low probability ($P(p\text{-}pair)<0.08$) of being the true parents (Table 2). Two individuals within habitat patch 30 could not be assigned to a parent–offspring relationship (Table 2). In habitat patch 37 ($P(ID)=9.42 \times 10^{-7}$), we found 24 putative parent–offspring relationships. Out of the 24 candidate parent relationships, 13 had a probability of being the true parents below 0.05, six had a probability between 0.05–0.5 and five had a probability greater than 0.5. One male and one female were candidate parents of two offsprings. Among the five putative offsprings, three were males and

two were females. One offspring was identified as a female from field evidences, as molecular sexing failed in this individual (Table 2). All individuals within habitat patch 37 were assigned to a possible parent–offspring relationship (Table 2). In habitat patch 417 (*P(ID)*=2.06 x 10^{-5}), we found eleven putative parent–offspring relationships and all had low probability (*P(p-pair)*<0.15). One individual within this habitat patch was not assigned to a parent–offspring relationship (Table 2).

Table 2: *Results of the parentage analysis using the program Parente (Cercueil et al. 2002) in the three habitat patches intensively studied. We report the number of unique genotypes identified,* n*, the estimated number of individuals,* N*, from Jacob et al. (submitted), and the probability of two individuals within local populations sharing the same genotype,* P(ID). *We report the offspring ID, the sex of the offspring determined by molecular markers, the candidate parent pair and the probability of the putative parents being the true parents* P(par–off). *We also indicate the number of individuals for which no putative parents were found,* Unrelated. *We report only parent–offspring relationships with* P(par–off) > *0.5.*

				Parentage analysis					
Patch ID	*n*	*N*	*P(ID)*	*Offspring ID*	*Sex*	*Mother*	*Father*	*P(par–off)*	*Unrelated*
p30	10	14	8.42 x 10^{-7}	-					2
p37	16	16	1.75 x 10^{-6}	Tu147	f[a]	Tu146	Tu141	0.68	0
				Tu149	f	Tu144	Tu151	0.53	
				Tu117	m	Tu161	Tu133	0.59	
				Tu157	m	Tu134	Tu156	0.88	
				Tu152	m	Tu146	Tu141	0.56	
p417	10	12	2.06 x 10^{-5}	-					1

[a] sex assessed from field evidence (see text for details)

Discussion

We combine genotyping of non-invasive samples and GIS as useful tools to monitor animal movements and to estimate the area of habitat used by individuals. We also show that microsatellite loci can be used to reconstruct parent–offspring relationships from a single sampling occasion. Thus, our results, although tentative, suggest that individual-based studies from non-invasively collected samples are feasible.

Population monitoring from non-invasive sample analyses

In the present study, we found two between-patch dispersal events in region B. Individuals moved from habitat patches 37 and 60, in which local capercaillie populations were estimated at N_{37}=16 and N_{60}=35 individuals (Debrunner et al. 2005; Jacob et al. submitted), to habitat patch 43 in which no evidence of a breeding population were found. Our results show that habitat patch 43 is visited, during the breeding season, by individuals from neighbouring habitat patches, which suggests that habitat patch 43 may act as a stepping-stone connecting habitat patches 37 and 60. Dispersal between local populations and habitat patches may explain the low genetic differentiation within region B reported in Jacob et al. (in prep). The largest observed dispersal distances within large and contiguous habitat patches were 1.5 and 1.8 km, *i.e.* close to the distance of 2 km assumed between neighbouring leks reported in Central Europe (Storch 1995){Regnaut, 2004 #518}, Scotland (Picozzi et al. 1992) and Norway (Rolstad & Wegge 1987). This coincidence suggests that these individuals may have visited different leks, which has been described for young males and females (Wegge & Larsen 1987; Storch 1997). Thus, our results indicate that individuals inhabiting habitat patches situated in mosaic landscape may disperse across larger distances during the breeding season than do individuals living in large and contiguous habitat patches.

Our study demonstrates that non-invasive samples may provide an alternative to telemetry-based studies to monitor individual dispersal within and among patches in rare and elusive species such as forest grouse. Knowing potential barriers to individual dispersal in the capercaillie may be valuable to elaborate management plans directed towards improving the connectivity among local populations.

We found no differences in the habitat use of individuals and dispersal distances among regions or between sexes. This result is not surprising given that our estimates represent the habitat use during the breeding season during the 2–3 days preceding the sampling.

Parentage analyses

We found candidate parent pairs with only a low probability of being the true parents in habitat patches 30 and 417. Three individuals within these habitat patches were unrelated to all other and each other in the patches, which suggest that these individuals were recent migrants that did not reproduce or that relatives of these individuals had not been sampled.

All the individuals identified in habitat patch 37, were identified either as reproducing adults or as offsprings and four different males were putative fathers. These results suggest that reproductive success may be distributed among several males and contrast with the view that one male may dominate the mating (Wegge & Larsen 1987). The number of chicks per female ranged from 1–2 (mean=1.25). Given that capercaillie females generally lay 6–8 eggs (Klaus et al. 1989), our results may indicate that juvenile mortality or emigration was high. We sampled 12 different individuals out of 16 estimated from non-invasive capture–mark–recapture analyses (Jacob et al. submitted). Thus, the risk exists that we missed some of the offsprings and that our sampling may not represent the true population. In the present study, we show that non-invasive sampling techniques and molecular genotyping can be used to investigate the mating system and breeding success in local populations of capercaillie, as reported in other species (Garnier et al. 2001; Griffith et al. 2002; Goossens et al. 2003; Hampton et al. 2004). In the capercaillie, males and females converge around lekking arenas during the breeding season. This period sees the highest densities of individuals in a restricted area, and a large proportion of the population can be sampled with limited effort. We therefore suggest that individual-based monitoring methods may be particularly relevant to investigate individual breeding success in lekking species like the capercaillie.

Potential limits of non-invasive studies

Several limitations appeared in this preliminary study, but they can be overcome by a careful sampling design and the use of large sets of polymorphic markers. In the present study, we did not aim at estimating the dispersal capacity and effective habitat use by individual capercaillie, but rather at assessing the feasibility of non-invasive sampling to conduct individual-based genetic studies in our study species. Therefore, our estimates of dispersal distances and habitat use should be regarded as tentative. Unrelated individuals were found in habitat patches 30 and 417, but not in habitat patch 37, in which sampling has been conducted across the entire patch by different people at different dates. These results indicate that conducting multiple sampling increases the probability to identify most of the individuals within local populations.

We could also show that our set of nine microsatellite loci had a limited power to conduct parentage analyses in isolated local populations in which the probability of identity among individuals' genotypes is relatively high, like those occupying habitat patches 30 and

417. Thus, increasing the number of polymorphic loci screened might be required if parentage analyses were to be conducted in those local populations.

References

Amos B, Schlotterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, 260, 670-672.

Bollmann K, Weibel P, Graf RF (2005) An analysis of central Alpine capercaillie spring habitat at the forest stand scale. *Forest. Ecol. Manag.*, 215, 307-318.

Bradley BJ, Vigilant L (2002) False alleles derived from microbial DNA pose a potential source of error in microsatellite genotyping of DNA from faeces. *Mol. Ecol. Notes*, 2, 602-605.

Cercueil A, Bellemain E, Manel S (2002) PARENTE: Computer program for parentage analysis. *J. Hered.*, 93, 458-459.

Debrunner R, Jacob G, Bollmann K (2005) Bestandesschätzung des Auerhuhns im Kanton St. Gallen mit genetischen Methoden. Projektschlussbericht. p. 27. Swiss Federal Research Institute WSL & Amt für Jagd und Fischerei St. Gallen.

Eggert LS, Eggert JA, Woodruff DS (2003) Estimating population size for elusive animals: the forest elephant of Kakum National Park, Ghana. *Mol. Ecol.*, 12, 1389-1402.

Frantz AC, Schaul M, Pope LC, Fack F, Schley L, Muller CP, Roper TJ (2004) Estimating population size by genotyping remotely plucked hair: the Eurasian badger. *J. Appl. Ecol.*, 41, 985-995.

Garnier JN, Bruford MW, Goossens B (2001) Mating system and reproductive skew in the black rhinoceros. *Mol. Ecol.*, 10, 2031-2041.

Gavin TA, Reynolds RT, Joy SM, Leslie D, May B (1998) Genetic evidence for low frequency of extra-pair fertilizations in northern Goshawks. *Condor*, 100, 556-560.

Goossens B, Setchell JM, Vidal C, Dilambaka E, Jamart A (2003) Successful reproduction in wild-released orphan chimpanzees (*Pan troglodytes troglodytes*). *Primates*, 44, 67-69.

Graf RF (2005) Analysis of capercaillie habitat at the landscape scale using aerial photographs and GIS. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Zurich.

Graf RF, Bollmann K, Suter W, Bugmann H (2004) Using a multi-scale model for identifying priority areas in capercaillie (*Tetrao urogallus*) conservation. In: *Proceedings of the 12th annual IALE(UK) conference "Landscape ecology of trees and forest"* (ed. Smithers R), pp. 84-90, Cirencester, UK.

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.*, 11, 2195-2212.

Hampton J, Pluske JR, Spencer PBS (2004) A preliminary genetic study of the social biology of feral pigs in south-western Australia and the implications for management. *Wildl. Res.*, 31, 375-381.

Helle P, Helle T, Lindén H (1994) Capercaillie (*Tetrao urogallus*) lekking sites in fragmented Finnish forest landscape. *Scand. J. For. Res.*, 9, 386-396.

Jacob G, Bollmann K, Schmid B, Gugerli F (in prep) Landscape barriers induce genetic structuring in the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps - implications for the management of the species.

Jacob G, Debrunner R, Gugerli F, Schmid B, Bollmann K (submitted) Estimating the population census sizes of capercaillie (*Tetrao urogallus* L., Aves) in the Swiss Alps from single-occasion genetic capture–recapture data. *Cons. Genet.*

Jenness J (2005) Convex hulls around points extension for Arcview 3.x, v. 1.2. Jenness Enterprises, Available at http://www.jennessent.com/arcview/convex_hulls.htm.

Kohn MH, Knauer F (1998) Phylogeography of Brown bear in Europe and excremental PCR the new tool in the genetic analysis of animals in the wild. *Ursus*, 10, 315-321.

Kohn MH, York EC, Kamradt DA, Haugt G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. *Proc. R. Soc. London Ser. B*, 266, 657-663.

Marti C (1986) Verbreitung und Bestand des Auerhuhns *Tetrao urogallus* in der Schweiz. *Ornithol. Beob.*, 83, 67-70.

Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecol. Applic.*, 10, 283-294.

Mollet P, Badilatti B, Bollmann K, Graf RF, Hess R, Jenny H, Mulhauser B, Perrenoud A, Rudmann F, Sachot S, Studer J (2003) Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. *Ornithol. Beob.*, 100, 67-86.

Morin PA, Wallis J, Moore JJ, Chakraborty R, Woodruff DS (1993) Noninvasive sampling and DNA amplification for paternity exclusion, community structure, and phylogeography in wild Chimpanzees. *Primates*, 34, 347-356.

Mowat G, Paetkau D (2002) Estimating marten *Martes americana* population size using hair capture and genetic tagging. *Wildlife Biol.*, 8, 201-209.

Navidi W, Arnheim N, Waterman M (1992) A multiple-tubes approach for accurate genotyping of very small DNA samples using PCR: statistical considerations. *Am. J. Hum. Genet.*, 50, 347-359.

Palsbøll PJ (1999) Genetic tagging: contemporary molecular ecology. *Biol. J. Linnean Soc.*, 68, 3-22.

Palsbøll PJ, Allen J, Berube M, Clapham PJ, Feddersen TP, Hammond PS, Hudson RR, Jorgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjonsson J, Sears R, Smith T, Sponer R, Stevick P, Oien N (1997) Genetic tagging of humpback whales. *Nature*, 388, 767-769.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci — a caution for parentage and population studies. *Mol. Ecol.*, 4, 249-252.

Petit E, Valière N (in press) Estimating population size: emphasis on noninvasive CMR. *Conserv. Biol.*

Picozzi N, Catt DC, Moss R (1992) Evaluation of capercaillie habitat. *J. Appl. Ecol.*, 29, 751-762.

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Mol. Ecol. Notes*, 1, 303-304.

R Development Core Team (2005) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rolstad J, Wegge P (1987) Distribution and size of capercaillie leks in relation to old forest fragmentation. *Oecologia*, 72, 389-394.

Rudnick JA, Katzner TE, Bragin EA, Rhodes OE, Dewoody JA (2005) Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Mol. Ecol.*, 14, 2959-2967.

Storch I (1995) Annual home ranges and spacing patterns of Capercaillie in Central Europe. *J. Wildlife Manage.*, 59, 392-400.

Storch I (1997) Male territoriality, female range use, and spatial organisation of capercaillie *Tetrao urogallus* leks. *Wildlife Biol.*, 3, 149-161.

Storch I (2000) Grouse Status Survey and Conservation Action Plan 2000-2004. (ed. Group WBSGS), p. 112 pp. IUCN, Gland, Switzerland and Cambridge, UK and the World Pheasant Association, Reading, UK.

Storch I (2001) *Tetrao urogallus* Capercaillie. *BWP Update*, 3, 1-24.

Taberlet P, Griffin S, Goosens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J (1996) Reliable genotyping of samples with very low DNA quantity using PCR. *Nucleic Acids Res.*, 24, 3189-3194.

Taberlet P, Luikart G (1999) Non-invasive genetic sampling and individual identification. *Biol. J. Linnean Soc.*, 68, 41-55.

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes*, 2, 377-379.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.*, 10, 249-256.

Wegge P, Larsen BB (1987) Spacing of adult and subadult male Common capercaillie during the breeding-season. *Auk*, 104, 481-490.

Wegge P, Rolstad J (1986) Size and spacing of capercaillie leks in relation to social-behavior and habitat. *Behav. Ecol. Sociobiol.*, 19, 401-408.

Wilson GJ, Frantz AC, Pope LC, Roper TJ, Burke TA, Cheeseman CL, Delahay RJ (2003) Estimation of badger abundance using faecal DNA typing. *J. Appl. Ecol.*, 40, 658-666.

Chapter 6

Synthesis

In the present chapter, I refer to my results and to published studies on related species to discuss the dynamics of the capercaillie in the Swiss Alps. Further, I elaborate recommendations for the management of the species in the Swiss Alps. Then, I describe how molecular techniques could be used to evaluate the success of management actions and to monitor the species. Finally, I identify technical and methodological limits of my study and point out, when possible, how these could be overcome in the near future.

*Population dynamics of the capercaillie (***Tetrao urogallus** *L.) in the Swiss Alps*

The distribution range of the capercaillie in the Swiss Alps contracted during the 20th century, with a more pronounced decline in the western part of the species' distribution range (Mollet et al. 2003). Simultaneously, the capercaillie disappeared from the lowlands and is nowadays restricted to mountainous areas. The remaining occurrences are separated from each other by mountain ranges higher than the natural range of the forest or large and continuous areas of unsuitable habitat. These landscape barriers greatly impact the exchange of individuals between regional populations and induce the genetic structuring of the capercaillie in the study area. I describe four regional capercaillie populations in the Swiss Alps and these are organised into a source–sink dynamics, as shown in other areas of the species distribution in the Alpine range (Segelbacher et al. 2003). The regional source population is in the Engadin Valley and may connect the Swiss capercaillie population with the Austrian population, which is considered as the core of the capercaillie metapopulation in central Europe (Segelbacher & Storch 2002). Regional populations are organised as network of local populations, which are sometimes restricted to a single breeding unit (lek). I observed

no genetic differentiation among local populations within regional populations, although some of the regional populations occupied landscapes with a considerable degree of habitat fragmentation. Thus, the population structure of the capercaillie in the Swiss Alps resembles a hierarchical structure.

I found contrasting patterns of genetic differentiation between regional populations, which suggests that gene flow between regional populations has been constrained. This hypothesis was confirmed by analysing past specimens dated before the pronounced and continuous decline of the capercaillie observed from 1950 onwards. One allele was found in past individuals from four regional populations, but absent in extant individuals from these regional populations. This indicates that allelic diversity has been lost following the decline of the capercaillie in the Swiss Alps.

Demographic estimates and viability of the capercaillie in the Swiss Alps

Mollet et al. (2003) estimated 450–500 capercaillie males in Switzerland, 375–425 of which are located in the Swiss Alps. Accounting for a balanced sex ratio, the census size of the population in the Swiss Alps should be around 750–850 individuals. This estimate is above the minimum population size of 500 individuals, a rule-of-thumb value used in conservation biology and proposed by Storch (1997) as first guideline for the conservation of the capercaillie. However, the "rule of 500" would hold true if the capercaillie was contiguously distributed. In the present study, I show that landscape barriers have induced genetic structure by constraining gene flow between regional populations. This suggests that the contraction of the species' distribution range constrained the genetic connectivity between regional populations. The census sizes within capercaillie regions 3, 4a, 4b and 5 described by Mollet et al. (2003) are estimated at 80, 115, 120–170 and 45–60 males, respectively. Using genetic capture–recapture analyses, I show that the field monitoring of the capercaillie likely underestimates the true numbers of individuals within local populations, especially so in the areas where the species' distribution is least known. Nevertheless, the census size of the total population may still be lower than the minimum size required to ensure the sustainability of the regional populations. Indeed, I found that regional populations lost genetic variability after landscape connectivity decreased. Genetic isolation increases the probability of mating between relatives and may have detrimental effects for the persistence of the regional populations. These effects were highlighted in a study by Westemeier et al. (1998) in Illinois, USA, who reported changes in the breeding success in an isolated population of the endangered Greater prairie-chicken, *Tympanuchus cupido pinnatus* Brewster. The authors

stress that the decline of the species primarily resulted from habitat loss, but was then induced by factors intrinsic to the breeding birds, *i.e.* the loss of individual fitness. Egg hatchability decreased with decreasing numbers of males counted on the leks, a proxy for the population census size, and this decrease was most pronounced when the number of breeding males dropped below 80. The population could only recover thanks to the translocation of unrelated birds from larger populations in neighbouring states. As pointed out above, two regional capercaillie populations in the Swiss Alps have 80 or less breeding male, one in the Engadin valley and the other in the central Prealps. The regional population in the Engadin has high level of genetic diversity and may still be connected to the Austrian population. In contrast, the regional population in the central Prealps is separated from the next regional population by large distances of unsuitable habitat. The local populations from this region showed the lowest level of genetic diversity. These results suggest that isolated regional populations may not be self-sustainable and may face a high risk of being negatively impacted by genetic factors if gene flow between them is not restored in the near future.

Implications for the conservation of the capercaillie in Switzerland

All the regional populations in the study area show evidence of recent decline (Mollet et al. 2003) and loss of connectivity (Graf 2005), which suggests that all of them require protection and management actions. I show that the capercaillie in the Swiss Alps is currently organised as source–sink population dynamics, and these dynamics would promote the long-term persistence of the species. Three out of four regional populations also show evidence of decline, especially in peripheral local populations. I therefore recommend that management actions focus on improving or restoring habitat within regional populations, which may have a positive impact on the population dynamics. Complementary measures should aim at maintaining or restoring the genetic connectivity between the regional populations by improving habitat patch sizes and quality along corridors between the regional populations. Segelbacher and Storch (2002) showed that the capercaillie metapopulation in central Europe is declining in the core and in peripheral areas of the distribution of the species. Given that the capercaillie population in the Swiss Alps is connected to the core of the Alpine metapopulation via the regional population of the Engadin Valley, the general decline of the capercaillie in central Europe may jeopardize the persistence of the capercaillie in the Swiss Alps. We therefore recommend that international collaborative efforts are undertaken to

identify the causes of the species' decline and to promote the recovery of the population in central Europe.

Non-invasive genetic monitoring as a tool to evaluate the success of management actions

In Chapter 2, I show that molecular studies provide a reliable tool to estimate the census sizes of local populations and may be less biased than field surveys in areas where the species distribution and the environmental factors explaining the species abundance are least known. Thus, non-invasive genetic studies may be used to monitor how local populations respond to management actions directed towards improving the quality of habitat patches. As discussed in Chapter 5, reconstructing parent–offspring relationships may provide valuable information on the mating system of the capercaillie and allow estimating the reproductive success and survival probability per age or sex class. Accurate estimates of population turnover are critical when evaluating the long-term viability of local populations. In addition, genetic studies allow monitoring individual's habitat use and tracking individuals' movements between local populations or forest patches. This provides a valuable alternative to telemetry studies to study the capercaillie's dispersal behaviour because capturing the individuals is no more necessary when conducting non-invasive genetic monitoring.

Thus, monitoring the capercaillie with non-invasive genetic techniques may prove to be useful to study the dynamics of the regional populations and the genetic connectivity between them.

Outlook of the study

I conducted genetic analyses at the individual scale (Chapters 2 and 5) and at the population level (Chapters 3 and 4) using twelve nuclear microsatellite loci. However, this set of markers gave limited information on the level of historical dispersal among local populations within and among regional populations. Owing to resource limitations, I restricted my analyses to screening microsatellite markers and did not study polymorphism at mitochondrial (mtDNA) sequences as planned initially. Sequencing the mtDNA control region could have provided additional information to infer the genetic connectivity between regional populations and to investigate the dispersal behaviour of males and females separately (Piertney et al. 2000; Johnson et al. 2003). As shown in chapter 5, non-invasive samples and genetic analyses could be used to collect data on individuals' breeding success and habitat use. Indeed, more information are necessary on population turnover and

individuals' dispersal behaviour to better understand the dynamics within capercaillie local populations and the factors affecting the connectivity among them. This approach would be most useful if estimates of the genetic connectivity among local populations was combined with habitat suitability maps inferred from habitat models (Graf et al. 2004).

This study was also limited by incomplete analyses of samples across the study area. Region S includes mountainous areas and is geographically isolated from regions A and region B by landscape barriers. This suggests that the population situated in region S may be genetically differentiated from those in regions A and B, and, thus, likely be considered as a fifth regional population. Given the central position of region S, analysing more individuals from this regional population would be required to infer the degree of connectivity between regions A, S and B, and to compare the level of genetic diversity among them.

Thus, I show that the capercaillie population in the Swiss Alps is becoming increasingly fragmented. The distribution range of the species is contracting, which decreases the connectivity between regional populations. Therefore, the capercaillie faces a high risk of being negatively impacted by genetic factors if the connectivity between regional populations is not restored.

References

Graf RF (2005) Analysis of capercaillie habitat at the landscape scale using aerial photographs and GIS. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Zurich.

Graf RF, Bollmann K, Suter W, Bugmann H (2004) Using a multi-scale model for identifying priority areas in capercaillie (*Tetrao urogallus*) conservation. In: *Proceedings of the 12th annual IALE(UK) conference "Landscape ecology of trees and forest"* (ed. Smithers R), pp. 84-90, Cirencester, UK.

Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology*, **12**, 3335-3347.

Mollet P, Badilatti B, Bollmann K, Graf RF, Hess R, Jenny H, Mulhauser B, Perrenoud A, Rudmann F, Sachot S, Studer J (2003) Verbreitung und Bestand des Auerhuhns (*Tetrao urogallus*) in der Schweiz 2001 und ihre Veränderung im 19. und 20. Jahrhundert. *Orn. Beob.*, **100**, 67-86.

Piertney SB, MacColl ADC, Bacon PJ, Racey PA, Lambin X, Dallas JF (2000) Matrilineal genetic structure and female-mediated gene flow in red grouse (*Lagopus lagopus scoticus*): an analysis using mitochondrial DNA. *Evolution*, **54**, 279-289.

Segelbacher G, Storch I (2002) Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. *Molecular Ecology*, **11**, 1669-1677.

Segelbacher G, Storch I, Tomiuk J (2003) Genetic evidence of capercaillie *Tetrao urogallus* dispersal sources and sinks in the Alps. *Wildlife Biol.*, **9**, 267-273.

Storch I (1997) The importance of scale in habitat conservation for an endangered species: the capercaillie in central Europe. In: *Wildlife in landscape ecology: effects of pattern and scale* (ed. Bissonette JA), pp. 311-330. Springer Verlag, New York.

Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695-1698.

Summary

Habitat deterioration and fragmentation affect the connectivity among populations by decreasing the size of habitat patches and increasing the distances between the remaining patches. These processes are therefore considered major threats for the persistence of species in altered landscapes. Small populations face a high risk of extinction due to stochastic events, *e.g.* when habitat patches are destroyed. In addition to this, intrinsic factors may impact the viability of small populations by decreasing individuals' survival and reproductive success, and limiting the capacity of populations to adapt to new environmental conditions. Thus, monitoring the population dynamics, *e.g.* by estimating the individual's fitness and the dispersal events between local populations, may be used to detect signs of population decline. However, monitoring rare or elusive species in the field require extensive human resources and bares the risk of further disturbing the individuals. Owing to the fast development of molecular methods in the last decade, it is nowadays possible to use non-invasive sampling, *e.g.* faeces and feather, as source of DNA for genetic studies. This sampling technique does not require to capture or even to observe the animals, which is particularly suitable for studying endangered and elusive species. Genetic studies conducted on different species showed that the inbreeding coefficient of individuals estimated at neutral loci correlates with individual's fitness. Similarly, the genetic differentiation between local populations reflects their connectivity. Thus, genetic studies can be used to estimate proxies for biological parameters to monitor the dynamics of population.

In the present study, I investigated different aspects of the dynamics of the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps that are relevant to the management of the species. For this, I analysed non-invasive samples at twelve nuclear microsatellite loci. First, genetic capture–recapture analyses showed that the field monitoring of the species tended to underestimate the number of individuals in the local populations. The genetic-based method is less influenced by the degree of knowledge of the study area and may therefore be used to evaluate how local populations respond to management actions. In a second part of my study, I describe four regional capercaillie populations. Continuous areas of unsuitable habitat, resulting from the recent contraction of the capercaillie's distribution range, and mountain ranges higher than the natural distribution of suitable habitat for the species separate the regional populations from each other. The regional populations are organised in a source–sink

constellation, in which we consider the regional population in the Engadin valley as the source for neighbouring regions. Within regional populations, local populations are not genetically differentiated. Thus, landscape barriers induce genetic structuring of the capercaillie in the Swiss Alps on a regional scale. Contrasting patterns of genetic diversity within and of genetic differentiation between regional populations suggest that their connectivity has been recently constrained. Indeed, museum specimens dated before 1950 show higher levels of genetic variability compared to extant populations from the same regions. I infer that regional populations became isolated and lost genetic variability through genetic drift following the species' decline in distribution and numbers since the 1950s. This suggests that regional populations may be impacted by genetic factors if the genetic connectivity between them is not restored. Based on these results, I recommend maintaining or improving the quality of habitat patches to allow local populations to increase in size. In addition, management actions should focus on restoring the genetic connectivity between regional populations to promote the sustainability of the capercaillie in the Swiss Alps. Finally, I show that non-invasive, individual-based monitoring of the capercaillie in the Swiss Alps is feasible and provides a valuable alternative to conventional field methods, such as telemetry or mark–recapture studies, to evaluate the success of management actions.

Zusammenfassung

Der Verlust und die Fragmentierung von Lebensräumen beeinflussen den Austausch von Individuen zwischen Populationen, indem sie die Größe der Lebensraum-Patches reduzieren und die Distanz zwischen ihnen erhöhen. Deshalb werden Verlust und Fragmentierung von Lebensräumen als wichtigste Gefährdungsfaktoren für die Überlebensfähigkeit von Arten in sich verändernden Landschaften angesehen. Kleine Populationen haben generell ein erhöhtes Aussterberisiko, da sie durch stochastische Ereignisse vollständig ausgelöscht werden können, zum Beispiel wenn ganze Lebensräume zerstört werden. Zusätzlich können intrinsische Faktoren die Überlebensfähigkeit und den Fortpflanzungserfolg von Individuen limitieren und die Anpassungsleistung von Populationen an neue Umweltbedingungen begrenzen. Es ist möglich, die Dynamik von Populationen zu überwachen, indem die individuelle Fitness und der Austausch von Individuen zwischen lokalen Populationen erhoben werden. Das feldgestützte Monitoring von seltenen oder heimlichen Arten ist allerdings arbeitsintensiv und erhöht die Störungen für die Tiere. Genetische Studien sind da eine willkommene Alternative. Die schnelle Entwicklung der molekularen Methoden in den letzten Jahren macht es heute möglich, nicht-invasive Proben wie Kot und Federn als DNA-Quellen zu benutzen. Dadurch müssen die Tiere weder gefangen noch beobachtet werden. Genetische Studien an neutralen Loci von verschiedenen Arten haben gezeigt, dass der Inzuchtkoeffizient mit der Fitness der Individuen korreliert. Ebenso ist die genetische Differenzierung zwischen Lokalpopulationen ein Mass für ihre Konnektivität. Entsprechend kann die Dynamik von Populationen überwacht werden, indem man einzelne Parameter mit genetischen Studien schätzt.

In der vorliegenden Studie habe ich verschiedene Aspekte der Dynamik des Auerhuhns (*Tetrao urogallus* L.) in den Schweizer Alpen untersucht, die für das Management dieser Art wichtig sind. Dazu habe ich nicht-invasiv gesammelte Proben mit zwölf nukleären Microsatelliten-Loci analysiert Die genetischen capture–recapture Analysen zeigten, dass die auf Spurentaxationen basierenden Bestandsschätzungen dazu neigen, die Zahl der Individuen in den lokalen Populationen zu unterschätzen. Die genetische Methode wird weniger durch Vorkenntnisse beeinflusst und kann deshalb für Erfolgskontrollen von Fördermassnahmen verwendet werden. Im zweiten Teil meiner Studie beschreibe ich vier regionale Auerhuhnpopulationen. Die Regionen sind durch Gebiete mit unbesiedelbarem Lebensraum voneinander getrennt, die durch Lebensraumveränderungen entstanden sind oder alpine

Regionen oberhalb der natürlichen Waldgrenze umfassen. Die regionalen Populationen sind in einer *source–sink* Konstellation organisiert, wobei die Population im Engadin eine *source* für benachbarte Regionen ist. Innerhalb regionaler Populationen unterscheiden sich die lokalen Populationen genetisch nicht. Entsprechend sind die Landschaftsbarrieren für die genetische Differenzierung der Auerhuhnpopulationen in den Schweizer Alpen auf der regionalen Ebene verantwortlich. Kontrastierende Muster der genetischen Diversität innerhalb und von genetischer Differenzierung zwischen regionalen Populationen weisen darauf hin, dass ihre Konnektivität begrenzt worden ist. So zeigen Museumpräparate aus der Zeit vor 1950 eine höhere genetischen Variabilität im Verglichen mit Proben von rezenten Populationen der gleichen Regionen. Daraus schließe ich, dass die regionalen Populationen einen Teil ihrer genetischen Variabilität durch genetischen Drift verloren haben, die durch den Rückgang der Verbreitung und des Bestands der Auerhühner seit den 1950er Jahren verursacht wurde. Dies zeigt, dass regionale Auerhuhnpopulationen durch genetische Faktoren negativ beeinflusst werden können, wenn die genetische Konnektivität zwischen ihnen nicht wieder hergestellt wird. Basierend auf diesen Resultaten empfehle ich, die Qualität der Lebensraum-Patches zu verbessern, um den Lokalpopulationen ein Bestandswachstum zu ermöglichen. Zusätzlich sollten Massnahmen für die Wiederherstellung der genetischen Konnektivität zwischen regionalen Populationen ergriffen werden, um die Überlebensfähigkeit der Auerhuhnpopulationen in den Schweizer Alpen zu fördern. Abschliesslich zeige ich, dass das nicht-invasive, individuenbasierte Monitoring des Auerhuhns in den Schweizer Alpen eine wertvolle Alternative zu herkömmlichen Methoden wie Telemetrie- oder *mark–recapture* Studien ist, die Wirkung von Schutzmassnahmen auf die Populationen zu untersuchen.

Résumé

La détérioration et la fragmentation des milieux entraînent la réduction de la taille des patches d'habitat et l'augmentation des distances entre eux, ce qui diminue la connectivité entre les populations. Ces processus sont donc considérés comme des menaces majeures pour le maintien des espèces dans les milieux dégradés. Les petites populations sont exposées à un fort risque d'extinction, par exemple en cas de destruction de l'habitat lors d'évènements stochastiques. De plus, des facteurs intrinsèques peuvent influencer la persistance de ces populations en diminuant la survie et le succès reproducteur des individus et en limitant la capacité d'adaptation de ces populations à des changements de conditions environnementales. L'étude du succès reproducteur des individus et des mouvements de dispersion entre populations, permet de détecter des signes éventuels de déclin des populations. Cependant, le suivi sur le terrain des espèces rares ou discrètes demande d'énormes moyens humains et augmente le risque de dérangement de la population. Grâce au développement rapide des méthodes de biologie moléculaire dans les dernières décennies, il est aujourd'hui possible d'utiliser des échantillons non-invasifs, par exemple des fèces ou des plumes, comme source d'ADN pour des études génétiques. Il n'est donc pas nécessaire d'observer ou de capturer les individus, ce qui est particulièrement adapté à l'étude d'espèces menacées et discrètes. Des études génétiques menées sur différentes espèces en utilisant des marqueurs génétiques dits neutres ont montré que le coefficient de consanguinité des individus est corrélé à leur fitness. De même, la différentiation génétique entre populations reflète leurs connectivités. L'estimation de paramètres biologiques par des techniques moléculaires permet ainsi d'étudier la dynamique des populations.

Dans cette étude, j'ai étudié différents aspects de la dynamique du Grand tétras (*Tetrao urogallus* L.) dans les Alpes suisses en rapport avec la conservation de l'espèce. Pour cela, j'ai analysé des échantillons récoltés non-invasivement avec douze marqueurs microsatellites nucléaires. L'analyse des données de capture–recapture ont montré que l'estimation des tailles de populations d'après des indices récoltées sur le terrain tendait à sous estimer le nombre d'individus dans les populations locales. La méthode génétique est moins influencée par le degré de connaissance des sites d'étude et peut donc être utilisée pour étudier la réponse des populations locales à des actions de protection. Dans la seconde partie de cette étude, je décris quatre populations régionales de Grand tétras. Ces régions sont séparées par des étendues continues d'habitat défavorable, résultant de la récente contraction

de l'aire de répartition de l'espèce, et des chaînes de montagne plus élevées que l'aire de répartition naturelle de l'habitat du Grand tétras. Les populations régionales sont organisées en une série de populations sources et puits, parmi lesquelles la population régionale de la vallée de l'Engadine apparaît comme la population source pour les Alpes suisses. A l'intérieur des régions, les populations locales ne sont pas différenciées génétiquement. Les barrières paysagères sont donc responsables de la structuration génétique à l'échelle régionale de la population de Grand tétras dans les Alpes suisses. La disparité entre la diversité génétique à l'intérieur des populations régionales et la différentiation génétique entre elles suggèrent que leurs connectivités ont été récemment contraintes. L'analyse de spécimens collectés dans des musées et datés d'avant 1950 révèle en effet des niveaux de variabilité génétique plus élevés que dans les populations actuelles provenant des mêmes régions. Suite au déclin de l'espèce depuis les années 1950, les populations régionales ont donc été progressivement isolées les unes des autres et ont perdu une partie de leur variabilité génétique. Ces résultats suggèrent que les populations régionales pourraient être affectées par des facteurs génétiques si la connectivité entre elles n'est pas restaurée. Je recommande donc que des mesures soient prises afin de maintenir ou d'améliorer la qualité de l'habitat et de permettre ainsi l'expansion des populations locales. De plus, ces mesures devraient viser à restaurer la connectivité génétique entre les populations régionales afin de promouvoir le maintien du Grand tétras dans les Alpes suisses. Finalement, je montre que l'étude génétique d'échantillons non-invasifs offre une précieuse alternative aux études de terrain conventionnelles, comme la télémétrie ou les programmes de marquage–recapture, pour étudier la biologie du Grand tétras et évaluer le succès des actions de protections.

Appendix I

Protocol for DNA extraction from faecal samples using the DNA Mini StoolKit (Qiagen, Hombrechtikon, Switzerland)

Procedures to control cross-sample contaminations are written in italic

Preparation of the samples

The preparation of the samples was carried out in a room free of capercaillie PCR-products.

a. Add 2.5 ml of buffer ATL (provided in the kit) in a 15-ml screw-locked plastic tube

Fill and label one tube per sample. Choose plastic tubes shorter than the pipette tips used to prevent cross-sample contamination by the pipette's body during liquid transfers.

b. Add 0.1–0.2 g of dessicated faecal samples (0.3–0.5 g for samples frozen undessicated)

Use gloves. Soak the working space with DNA-Off solution (or javel-water). Cut and weigh samples on a 10x10 cm aluminium sheet. Change gloves and scalpel blades, and soak the working space and forceps between samples.

c. Vortex gently the tubes and store overnight (8–12 hours) at room temperature in a place protected from direct light.

DNA extraction

DNA extractions were conducted in a dedicated room, free of PCR products. Samples were processed in a hood, under constant outward air-flow. The centrifuge and vortex were exclusively used for DNA extraction and cleaned after each extraction session. Pipette tips, 1.5 and 2-ml tubes were stored in an environment free of PCR product and brought directly to the extraction room.

For every samples, we prepared and labelled five plastic tubes:

Tube #1: 2-ml tube, empty

Tube #2: 2-ml tube + 1 InhibitEx tablet (provided in the kit)

Tube #3: 1.5-ml tube, empty

Tube #4: 2-ml tube + 180 µl AL buffer + 20 µl Proteinase K (mg/ml)

Tube #5–9: 2-ml collection tubes (provided in the kit)

Tube #10: 1.5 ml tube, empty

1. Vortex shortly to release the epithelial cells lining from the surface of the samples.

   Do not disintegrate the sample to avoid too many inhibitors in the solution.

2. Transfer the supernatant in tube #1 and centrifuge 10 min at 16000 g (full speed) to pellet the particles.
3. Transfer 1.4 ml in tube #2 and vortex vigorously until the tablet is completely suspended.
4. Incubate the suspension for 1 min at RT to allow inhibitors to adsorb to the InhibitEX matrix.
5. Centrifuge full speed for 10 min at 16000 g, to pellet particles and inhibitors bound to InhibitEX.
6. Immediately after the centrifuge stops, pipette all of the supernatant into tube #3.
7. Centrifuge tube #3 10 min at 16000 g, transfer 600 µl of the supernatant to tube #4.
8. Add 600 µl of buffer AL (Qiagen, Hombrechtikon, Switzerland), immediately vortex (15 sec) and incubate at 70°C for at least 10 min.
9. Add 600 µl of 100 % EtOH into tube #4 and vortex 15 sec.
10. Carefully apply 600 µl from tube #4 into a QIAamp spin column (provided in the kit).

    Centrifuge at 16000 g for 2 min and discard the collection tube.

    Place the spin column on collection tube #5.

11. Repeat step 10 with collection tube #6.
12. Repeat step 10 with collection tube #7.
13. Add 600 µl of AW1 to the column,

    Centrifuge 2 min at 16000 g and discard the collection tube.

    Place the column on collection tube #8.

14. Add 600 μl of AW2 to the column.

    Centrifuge 6 min at 16000 g and discard the collection tube.

15. Place the spin column in tube #9.

    Centrifuge 2 min at 16000 g.

    Discard the collection tube containing the filtrate.

16. Transfer the spin column into tube #10 and pipette 75 μl buffer AE.

    Incubate for 1 min at RT.

    Centrifuge 2 min at 16000 g, to elute the DNA.

17. Repeat step 16 with another 75 μL buffer AE.

DNA extraction from feather samples with the TissueKit (Qiagen, Hombrechtikon, Switzerland)

Buffers ATL, AL, AW1, AW2 and AE are provided in the kit

Collection tubes are provided in the kit

For every samples, we prepared and labelled five tubes:

Tube #1: 2 ml, 200–600 µl buffer ATL + 20 µl proteinase K (mg/ml)

Tubes # 3–6: collection tubes (provided in the kit)

1. Cut 0.5 to 1 cm of the tail of the feather into small pieces,

   Place in tube #1 and vortex thoroughly,

   Incubate at 55° overnight in a shaking water bath/hybridisation oven.
2. Vortex for 15 sec,

   Add 200–600 µl of buffer AL,

   Vortex and incubate at 70°C for 10 min.
3. Add 200–600 µl EtOH 100%,

   Vortex thoroughly.
4. Load 600 µl into a Qiaquick column + collection tube,

   Centrifuge at 6000 g for 1 min,

   Discard the flow-through and the collection tube.
5. If necessary, repeat step 4 up to loading the total liquid in tube #1.
6. Place the column into collection tube #4,

   Add 500 µl buffer AW1,

   Centrifuge at 6000 g for 1 min,

   Discard the flow-through and the collection tube.

7. Place the column into collection tube #5,

   Add 500 µl buffer AW2,

   Centrifuge at full speed for 3 min,

   Discard the flow-through and the collection tube.

8. Place the column into collection tube #6,

   Transfer 75 µl buffer AE directly to the membrane,

   Incubate at RT for 1 min,

   Centrifuge at 6000 g for 1 min.

9. Transfer 75 µl buffer AE directly to the membrane,

   Incubate at room temperature for 1 min,

   Centrifuge at 6000 g for 1 min.

10. Discard the column.
11. Store DNA extracts at 5°C if PCR are to be run in the next days, store at -20°C otherwise.

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Curriculum Vitae

Personal information

Name	Jacob
Surname	Gwenaël
Date of birth	29th May 1977, in France
Nationality	French

Education

Science Degree 1995–1997	Biology with minor on Environmental Factors and Biology University Joseph Fourier, Grenoble, France
Master's Degree 1997–2001	Biology of Populations and Ecosystems, minor on Management of Natural Heritage University Joseph Fourier, Grenoble, France
PhD title	Conservation genetics of the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps Swiss Federal Research Institute WSL/ University of Zurich
PhD supervisor	Prof. Dr. Bernhard Schmid